CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 20-986

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Clinical Pharmacology and Biopharmaceutics Review

NDA:	**20-986**
Insulin Aspart for Injection (100 U/ml) (NovoLog®)	
Submission Date:	**15 September 1998** **8 October 1998** **20 October 1998** **8 March 1999** **20 April 1999** **13 May 1999** **25 May 1999** **26 May 1999**
Type of Submission:	**New Drug Application (1-S)**
Reviewer:	**Michael J. Fossler**

JUL 20 1999

Synopsis

Novo Nordisk has submitted NDA 20-986 for insulin aspart, an analog of human insulin. The proposed indication is for the treatment of diabetes mellitus. Insulin aspart (Novolog) has a quick onset of action and a short duration of activity, and is intended to be given within — minutes of a meal.

The bioavailability of insulin aspart (IAsp) was compared with that of human insulin in a randomized crossover trial in 24 healthy volunteers. The results appear to indicate that the overall extent of absorption of IAsp may be greater than human regular insulin; however, this is more likely due to incomplete absorption of regular insulin due to the truncation of sampling after 6 hours. A study was also performed comparing insulin aspart to the approved insulin analog on the market, insulin lispro (Humalog/Lilly). The study showed that the two insulins are absorbed to a similar extent (90% confidence intervals of the ratio of AUC 0.85-0.95), but lispro had a slightly higher peak (26.2 vs 30.5, 90% CI 0.77-0.95). The median time to peak for both insulins were similar at 40 minutes. During the development of insulin aspart, ——— ——————————————————— . A bioequivalence study (Study 044) was performed in order to assess the impact of this change on the rate and extent of absorption of insulin aspart. The two formulations were found to be bioequivalent (90% CI for AUC: 91.6-110, for Cmax: 88.6-101).

To determine whether the fast absorption of insulin aspart is preserved when mixed with NPH, arandomized crossover bioequivalence trial was performed in 28 healthy volunteers. The results showthat the overall extent of absorption of insulin aspart is unaffected; however, there does appear to besome minor attenuation of the peak. As expected, the time to peak is also affected to a minor extent.Interestingly, it appears that the peak of NPH is slightly increased upon mixing by the same extent, which suggests that some exchange is taking place between the NPH crystals of human insulin and thesoluble human insulin. These findings suggest that insulin aspart may be mixed in the same syringe with Novo's NPH immediately before injection with only a minor delay in absorption (6-29%), but that the potential for severe attenuation of its absorption rate may occur if injection is delayed. No data exist on the effect of mixing insulin aspart with crystalline zinc insulins such as Lente or UltraLente.

To determine whether insulin aspart is subject to site effects, a study was performed in 18 healthy volunteers in which either human insulin or insulin aspart was given by sc injection in the abdominal, deltoid or thigh regions. The results indicate that insulin aspart is absorbed faster than human insulin at each site (abdomen, femoral, deltoid) tested.

The pharmacokinetics of insulin aspart has been studied in normal volunteers and in patients with Type 1 diabetes. In both populations, peak concentrations of insulin aspart are reached sooner (40 minutes vs. 120 minutes) and are higher (40.9 vs. 17.5 mU/l) than human insulin. After intravenous administration, the clearance of insulin aspart (1.22±0.32 L/hr/kg) is nearly identical to that of human insulin (1.24±0.118 L/hr/kg). In healthy male volunteers, the intra- and inter-subject variability of the pharmacokinetics of insulin aspart was similar to that of human insulin except for Cmax, which appeared to have higher intra-subject variability, but lower intersubject variability than human insulin.

A study was performed in Type 1 diabetic males given either insulin aspart just before a standard meal, human soluble insulin given 30 minutes prior the meal, or human soluble insulin given just after the meal. As in the healthy volunteer studies, Cmax was significantly higher (82.1 vs 35.9, 39.9) and occurred significantly sooner than human insulin given at the time of the meal (39.1 min vs. 100.7 min).The time to peak for insulin aspart and human insulin given 30 minutes prior to a meal were similar(39.1 min vs. 39.9 min post-meal), which is expected, since it is generally recommended to patients that they dose themselves with regular insulin 30 minutes prior to eating. No studies were performed in Type 2 patients.

Studies in renally or hepatically impaired patients were not performed. One trial that enrolled both men and women showed no significant difference in the pharmacokinetics of insulin aspart in men and women when body weight was taken into account. Studies in children aged 7-12 and 13-17 showed that insulin aspart is absorbed faster than human insulin, and behaves qualitatively similar to that in adults.

The pharmacokinetics and pharmacodynamics of insulin aspart were studied in normal volunteers using the glucose clamp technique. In normal volunteers, the peak glucose infusion rate (GIR) is somewhat higher (mean of 1026 mg/min vs. 959 mg/min, p=0.09) and is reached sooner (median 127 min vs. 182min., p=0.048) after insulin aspart administration as compared with human insulin. Overall, the peak GIR after insulin aspart administration is reached slightly, but significantly faster than that after human insulin administration.

One study examined the PK/PD of insulin aspart in Type 1 patients. The euglycemic clamp was not used. Instead, each subject was given a standard meal with doses of insulin aspart given immediately before eating. Human insulin was given either 30 minutes prior to eating (the standard practice) or immediately before (what patients usually do). The results clearly show that insulin aspart, because of its faster absorption, significantly attenuates the post-prandial plasma glucose peak as compared with human insulin given either 30 minutes prior to eating or immediately before eating. This study also demonstrates that the duration of action of insulin aspart is significantly shorter than human insulin. No data were provided on the PK/PD of insulin aspart in Type 2 patients.

Recommendations

The clinical pharmacology and biopharmaceutics portion of NDA 20-986 is approved provided that the labeling changes indicated are made. The firm is asked to provide a commitment to perform the Phase 4 studies as outlined in **Comments to the Firm.**

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Appendix of Study Summaries (available from DPE-2 upon request)

I. Background

Novo Nordisk has submitted NDA 20-986 for insulin aspart, an analog of human insulin. Insulin aspart is identical in structure to human insulin except that at position 28 on the B chain the native proline residue is replaced with aspartic acid. This substitution allows the compound to exist as a monomer insolution, in contrast to human insulin, which exists as a hexameric form. Thus, the analog is absorbed more rapidly after subcutaneous injection.

Insulin aspart is a 2-chain peptide containing 51 amino acids. It is insoluble in organic solvents, and in aqueous solutions of pH ~ 5.1. In aqueous solutions of pH < 3.5 or above 6.5, the solubility is greater than 25 mg/ml.

The specific questions to be answered for this compound are the following:

1) How does the pharmacokinetics and pharmacodynamics of insulin aspart compare with that of soluble human insulin?
2) How does the alteration in structure affect the inter- and intra-patient variability of the the absorption kinetics of insulin aspart, and how do these parameters compare with human insulin?
3) What effect do diseases such as renal or hepatic impairment have on the disposition of insulin aspart?
4) What effect does the site of injection have on the pharmacokinetics of insulin aspart?
5) What effect does mixing insulin aspart with NPH have on its absorption kinetics and overall bioavailability?
6) When should insulin aspart be given relative to mealtime?

II. Assay Method and Validation

III. Bioavailability and Bioequivalence

Relative Bioavailability

The bioavailability of insulin aspart was compared with that of human insulin in a randomized crossover trial in 24 healthy volunteers. The results, shown in Table 2, appear to indicate that the overall extent of absorption of IAsp may be greater than human regular insulin; however, this is more likely due to incomplete absorption of regular insulin due to the truncation of sampling after 8 hours. As expected, the reduced tendency toward hexamer formation results in faster absorption, as shown by the shorter time to peak, and the higher Cmax. Figure 1 shows a plot of the mean insulin concentrations over time for the two treatments.

Table 1: Comparison of IAsp and human insulin pharmacokinetics in normal volunteers (Study 022). Values in the table are mean" "SD (range) except where noted.

Parameter	Insulin Aspart	Human Insulin
AUC(0-480 min) (mU*min/L)	6461±1207 (4594-9155)	4669±836 (3066-6644)
95% CI of the ratio (IAsp/HI)	**1.38 (1.24- 1.54)**	
Cmax (mU/L)	40.9±11.2 (23.3-64.6)	17.5±4.3 (10.7-26.7)
95% CI of the ratio (IAsp/HI)	**2.31 (2.02-2.65)**	
‡Tmax (min)	40	120

‡Median (min, max)

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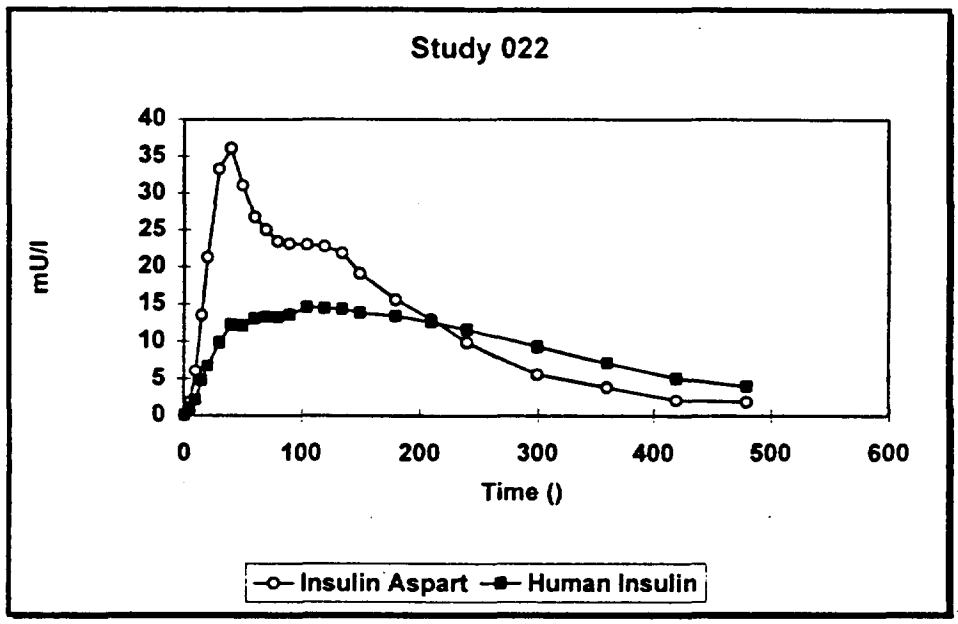
Figure 1: mean insulin concentration vs. time profiles for Study 022

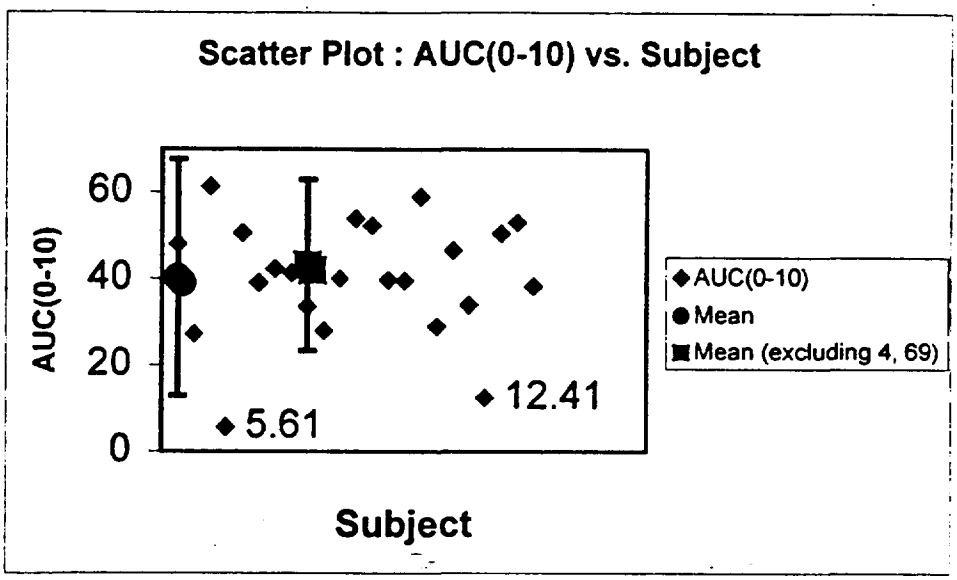


A study was also performed comparing insulin aspart to the approved insulin analog on the market, insulin lispro (Humalog/Lilly). The study showed that the two insulins are absorbed to the same extent (90% confidence intervals of the ratio of AUC 0.85-0.95), but lispro had a slightly higher peak (26.2 vs 30.5 mU/L, 90% CI 0.77-0.95). The median time to peak for both insulins were similar at 40 minutes.

Bioequivalence

During the development of insulin aspart, [ ]. A bioequivalence study (Study 044) was performed in order to assess the impact of these changes on the rate and extent of absorption of insulin aspart. The study was performed in 24 healthy volunteers in randomized crossover fashion. Initially, the two products appeared to be inequivalent; however, looking at the data, it appeared that 2 subjects (#4, 69) did not receive a full dose during the test arm (Figure 2). Subsequently, the injection devices used for these two subjects were investigated and found to be malfunctioning. When these subjects were excluded, the two formulations were found to be bioequivalent (90% CI for AUC: 91.6-110, for Cmax: 88.6-101).

Figure 2: Scatter plot of AUC vs. subject along with means±2SD both with and without subjects 4 and 69. These two subjects (identified by AUC values next to data points) clearly show that the two subjects are outliers. It also shows that deleting these two subjects does not affect the estimate of the mean to a great extent, but does affect the SD.



Effect of Mixing

Many diabetics take both long-acting basal insulins such as NPH, along with shorter-acting insulin. It is often more convenient for the patient to mix the two insulins in the same syringe and inject them together. To determine whether the fast absorption of insulin aspart is preserved when mixed with NPH, a randomized crossover bioequivalence trial was performed in 28 healthy volunteers. A insulin aspart-specific (ie, no cross-reaction with human insulin) assay was used in this trial, as well as an assay that measured both insulin aspart and human insulin. The data for the insulin aspart-portion of the analysis is shown below in Table 2.

Table 2: Results of the insulin aspart portion of the mixing study. Values in the table are mean±SD (range) except where noted.

Parameter	Mixed	Separate
AUC(0-24) (mU*hr/L)	41.9±17.08 (20.1-107)	41.9±17.08 (20.1-107)
90% CI	*0.99 (0.87 – 1.12)*	
AUC(0-inf) (mU*hr/L)	41.9±17.08 (20.1-107)	41.9±17.08 (20.1-107)
90% CI	**0.99 (0.87 – 1.12)**	
Cmax (mU/L)	18.7±7.5 (5.2-36.5)	23.5±9.9 (7.3-53.8)
90% CI	**0.81 (0.71-0.94)**	
‡tmax (min)	45	50

‡Median (range)

The results show that the overall extent of absorption of insulin aspart is unaffected; however, there does appear to be some minor attenuation of the peak, with a commensurate minor change in tmax. Interestingly, it appears that the peak of NPH is slightly increased upon mixing by the same extent (mean ratio of Cmax of NPH and 90% CI: 1.19 (1.02-1.39)), which suggests that some exchange is taking place between the NPH crystals of human insulin and the soluble insulin aspart. These findings suggest that insulin aspart may be mixed in the same syringe with Novo's NPH immediately before injection with only a minor delay in absorption (6-29%), but that the potential for severe attenuation ofits absorption rate may occur if injection is delayed. No data exist on the effect of mixing insulin aspart with crystalline zinc insulins such as Lente or UltraLente.

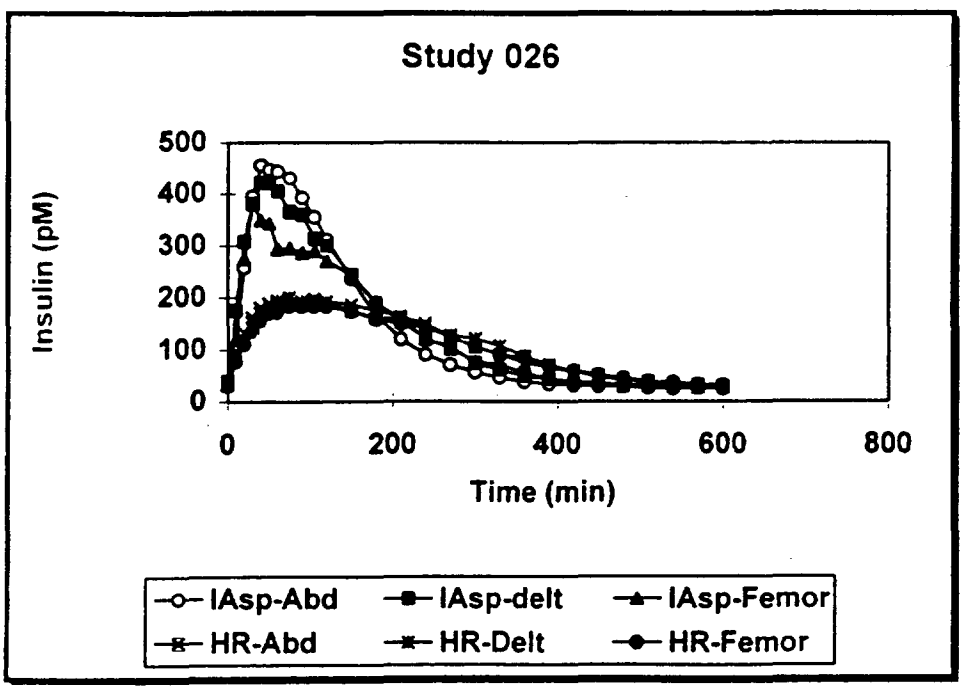
Effect of Injection Sites

The site of injection has a profound effect on the rate of absorption of insulin. It is generally accepted that the rate of absorption of human insulin is fastest from the abdomen, intermediate from the deltoid region, and slowest from the thigh. To determine whether insulin aspart is subject to site effects, a study was performed in 18 healthy volunteers in which either human insulin or insulin aspart was given by sc injection in the abdominal, deltoid or thigh regions. The results (figure 3) indicate that insulin aspart is absorbed faster than human insulin at each site.

IV. Metabolism

No metabolism studies were performed. It is likely that insulin aspart is metabolized similar to human insulin.

Figure 3: Serum Insulin vs. time profiles for human insulin and insulin aspart after a 0.2 U/kg injection in the abdominal, deltoid, or femoral regions (Study 026)



V. Pharmacokinetics

Normal Volunteers

Table 3 contains data from Study 052, which was performed in normal volunteers. This study used the aspart-specific assay, so that the mean half-life (t½) could be estimated. As expected, the t½ is short, less than 1 hour, so that a given dose will be essentially completely eliminated after about 4 hours. In a study in which both human insulin and insulin lispro were given intravenously, the clearance of insulin aspart (1.22±0.32 L/hr/kg) was nearly identical to that of human insulin (1.24±0.12 L/hr/kg).

Table 3: Single-dose Pharmacokinetics of *insulin aspart in normal volunteers given s.c. Under euglycemic clamp conditions

Parameter	Mean ± SD
AUC(0-24) (mU*hr/L)	44.3 ±20.21 (16.6-114)
AUC(0-inf) (mU*hr/L)	44.3±20.21 (16.6-114)
Cmax (mU/L)	23.5±9.9 (7.3-53.8)
tmax (min)	57±16.2
t½ (hours)	0.71±0.28 (0.46-1.41)

*data from separate injection arm only, insulin aspart-specific assay used.

A study was performed in normal volunteers to examine the intra- and inter-subject variability of insulin aspart as compared with soluble human insulin. Eighteen male volunteers were randomized to receive four successive doses of either insulin aspart or human insulin under euglycemic clamp conditions. Mixed-effect modeling was used to compute both intra and inter subject variability of both PK and PD parameters. The variability estimates were then compared by F-test. Non-parameteric tests were used to explore the inter-and intra-subject variability in tmax. A summary of the results is presented in Table 4. The results indicate that peak insulin aspart levels are more variable than human insulin within subject, but less variable between subjects. The results of the nonparametric analysis of tmax indicated that the inter-subject variability was similar for the two insulins, but the intra-subject variability for insulin aspart is less that that of human regular insulin ($p < 0.05$). The clinical utility of this information is unclear, since the study was performed in normal volunteers and only men were enrolled.

Table 4: Inter-and intra-subject variability estimates of insulin aspart and human soluble insulin given to healthy male volunteers. AUGC – area under the glucose curve. TAUC½ - median of the insulin plasma curve.

Parameter	Type of Variabilit	σ^2_{IAsp}	σ^2_{HI}	p
AUC	Intra-subject	3.24	2.79	ns
	Inter-subject	1.79	1.84	ns
Cmax	Intra-subject	822	67.4	<0.0001
	Inter-subject	2.97	24.02	0.0051
MRT	Intra-subject	239	270	ns
	Inter-subject	116	437	ns
AUGC	Intra-subject	0.407	0.337	ns
	Inter-subject	0.325	0.390	ns
GIRmax	Intra-subject	8.79	6.09	ns
	Inter-subject	6.16	3.3	ns
TAUC½	Intra-subject	427	681	ns
	Inter-subject	274	484	ns

Patients

A study was performed in Type 1 diabetic males given either insulin aspart just before a standard meal, human soluble insulin given 30 minutes prior the meal, or human soluble insulin given just after the meal. As in the healthy volunteer studies, Cmax was significantly higher (82.1 vs 35.9, 39.9) and occurred significantly sooner than human insulin given at the time of the meal (39.1 min vs. 100.7 min). The time to peak for insulin aspart was and human insulin given 30 minutes prior to a meal were similar (39.1 min vs. 55.2 min), which is expected, since it is generally recommended to patients that they dose themselves with regular insulin 30 minutes prior to eating.

Comparing the pharmacokinetics of insulin aspart in Type 1 patients and normal volunteers is difficult since there is no specific study of the two populations. However, examining Table 3, one can see that in both groups, insulin aspart peaks at about 40-50 minutes. Since this patient study used a much higher insulin dose (0.15 U/kg) than the healthy volunteer studies (0.06-0.1 U/kg), and since insulin pharmacokinetics may be non-linear within this range, cross-study comparison of exposure is not possible.

VI. Special Populations

Hepatic, Renal

There are no studies with insulin aspart. It is known that patients with hepatic or renal impairment must be carefully monitored.

Age

There are no data on the effect of old age on the pharmacokinetics of insulin aspart. See *Pediatrics* for data in children

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Gender

Study 052 enrolled both men and women in the trial. Men had higher serum insulin aspart concentrations (AUC 52.7 vs. 35.2) due to dosing by body weight. This difference disappeared (0.67 men vs. 0.60 women) when AUC values were normalized for body weight. The half-life in men (median 0.74 hr) and women (median 0.70 hr) suggest that there is no difference in clearance of insulin aspart between the genders. The similarities in tmax (median tmax in men 55 min, women 50 min) suggest that there is no difference between the genders with regard to absorption rate.

Pediatrics

A single-dose PK study was performed in children aged 7-12 (n=9) and 13-17 years (n=9). Insulin aspart showed a similar pattern of absorption in both age groups as compared with human insulin, with peak levels occuring at a median time of 40 minutes in both groups as compared with human insulin (median tmax 70-75 minutes).

VII. Drug Interactions

No studies were performed.

VIII. Pharmacokinetic/Pharmacodynamic Relationships

Normal Volunteers

The pharmacokinetics and pharmacodynamics of insulin aspart were studied in normal volunteers using the glucose clamp technique. In this technique, the glucose infusion rate required to maintain euglycemia is used as a biological marker of drug action. From Figure 4, it can be seen that in normal volunteers, the peak GIR is somewhat higher (mean of 1026 mg/min vs. 959 mg/min, p=0.09) and is reached sooner (median 127 min vs. 182 min., p=0.048) after insulin aspart administration as compared with human insulin. Overall, the peak GIR after insulin aspart administration is reached slightly, but significantly faster than that after human insulin administration.

Type 1 Patients

One study examined the PK/PD of insulin aspart in Type 1 patients. The euglycemic clamp was not used. Instead, each subject was given a standard meal with doses of insulin aspart given immediately before eating. Human insulin was given either 30 minutes prior to eating (the standard practice) or immediately before (what patients usually do). The results, shown in Figure 5, clearly show that insulin aspart, because of its faster absorption, significantly attenuates the post-prandial plasma glucose peak as compared with human insulin given either 30 minutes prior to eating or immediately before eating. The figure also shows, in a compelling way, that the duration of action of insulin aspart is significantly shorter than human insulin.
A study was also performed in pediatric diabetics; however, because the meals were not standardized, the glucose profiles are not very useful. The pharmacokinetics of insulin aspart showed the expected increase in absorption rate, as previously discussed.

Type 2 Patients

A study was performed in Type 2 patients, but the data were not analyzed, due to technical problems.

Figure 4: Mean GIR vs. time in 24 healthy male volunteers given either human insulin or insulin aspart

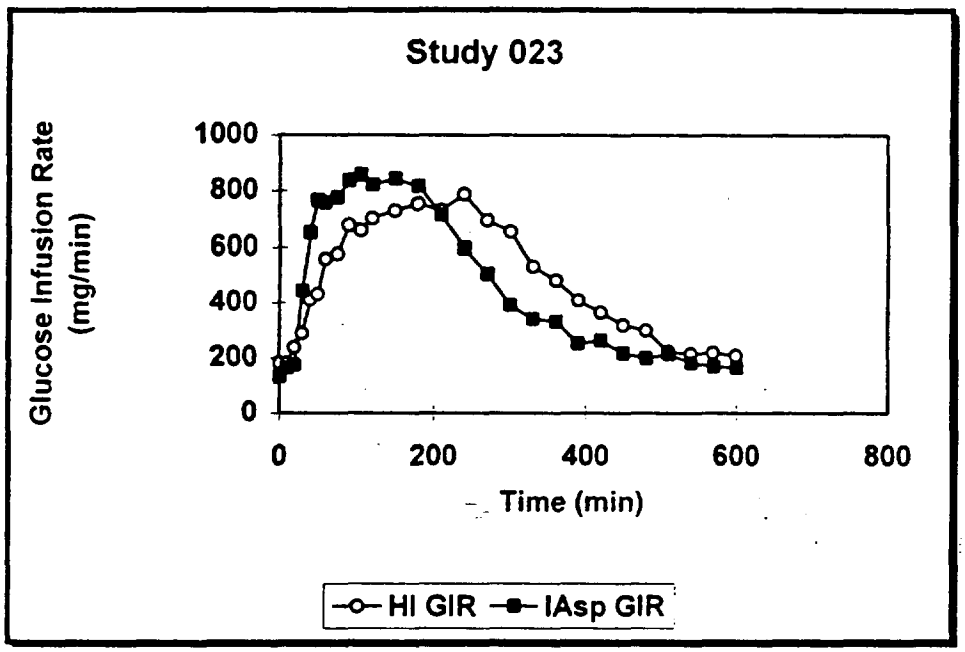
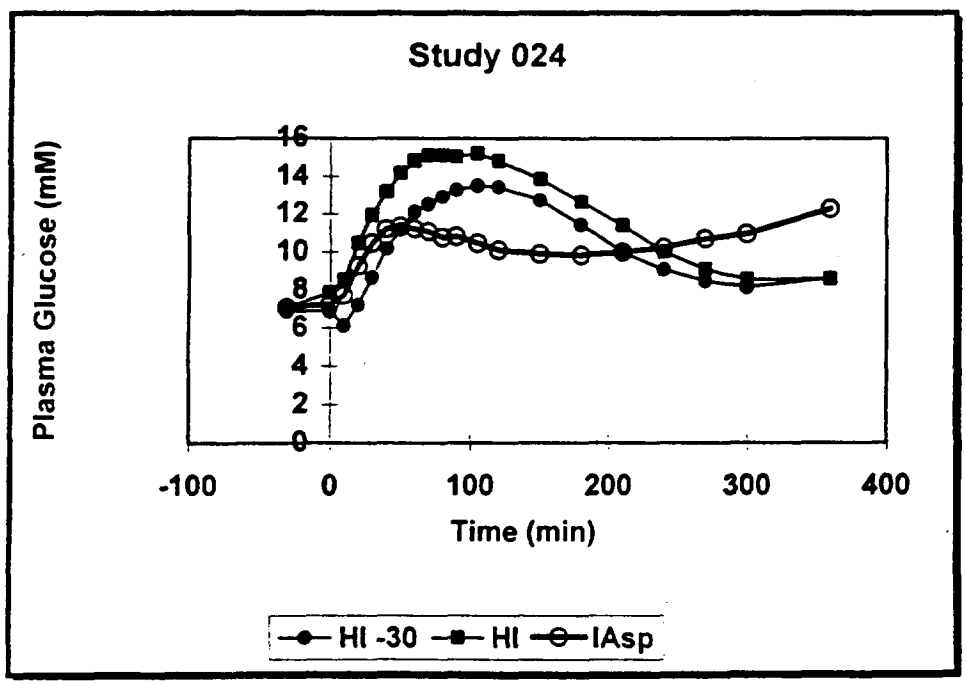


Figure 5: Mean plasma glucose levels after a standard breakfast in 22 Type 1 male subjects with diabetes given insulin aspart or human insulin immediately before a meal, or human insulin 30 minutes prior to eating.



IX. Dosage and Administration

The dose of insulin aspart to be given to each patient must be highly individualized based on the patient's current disease state, degree of glucose control, and other patient-related variables.

X. Formulation

The formulation is listed in Table 5. Insulin aspart will be marketed in both auto-injectors (NovoPen®) and in 10mL vials. No specific studies were performed comparing the two modes of administration.

Table 5: To-be-marketed formulation.

Ingredient	Amount per mL
Insulin Aspart	[redacted]
Glycerin	16 mg
Phenol	1.5 mg
m-cresol	1.72 mg
Zinc	19.6 mg
Na_2HPO_4	1.25 mg
NaCl	0.58 mg

XI. Reviewer Conclusions

- Compared with human insulin, peak concentrations are higher and occur earlier with insulin aspart. Metabolic clearance for insulin aspart is similar to human insulin at equivalent doses.

- Under euglycemic clamp conditions, peak GIR is higher and reached earlier with insulin aspart as compared with human insulin. In patients given a standardized meal, the post-prandial blood glucose peak is significantly attenuated as compared to human insulin. Glucose levels begin to rise 3-4 hours post-injection, demonstrating the shorter duration of action of insulin aspart as compared with human insulin.

- Within-subject variability in peak insulin aspart concentrations are higher than that seen after human insulin administration. In contrast, between-subject variability is smaller after aspart administration as compared with human insulin. For other PK parameters, the inter and intra-subject variability does not differ.

- Studies in patients with renal or hepatic disease were not performed.

- At all of the common injection sites, insulin aspart maintains its faster absorption as compared with human insulin.

- Mixing insulin aspart with NPH just before injection does not appear to affect its absorption rate. No data exist on mixing insulin aspart with other long-acting insulins such as crystalline zinc preparations.

- Insulin aspart should be given just before a meal.

XII. Comments to firm

1) The firm is requested to commit to performing Phase 4 studies in the following special populations in order to determine the impact on the PK/PD of insulin aspart:

- Renally and hepatically-impaired patients
- Obese vs. thin patients

XIII. Labeling Comments

Labeling comments are shown below. ~~Strike-out~~ formatting is used for text which should be deleted. ***Bold italic*** indicates additions/modifications.

1) CLINICAL PHARMACOLOGY

Mechanism of Action – no changes

Pharmacokinetics

Pharmacodynamics

Special Populations

Age and Gender

Gender- ***In healthy volunteers, no difference in insulin aspart levels were seen between men and women when body weight differences were taken into account. There was no significant difference in efficacy noted (as assessed by HbA_{1c})*** between genders in a trial in Type 1 diabetic patients.[60]

Ethnic origin- The effect of ethnic origin on the pharmacokinetics of insulin aspart has not been studied.

[

]

Smoking- The effect of smoking on the pharmacokinetics/ pharmacodynamics of ——— has not been studied.

2) Information for Patients

Mixing of insulins

[

]

XIV. **Signatures**

/S/ 7/20/99

Michael J. Fossler, Pharm.D., Ph.D.

Division of Pharmaceutical Evaluation II
Office of Clinical Pharmacology and Biopharmaceutics

RD initialed by Hae-Young Ahn, Ph.D., Team Leader /S/ 7/19/99
FT

version: Final

Briefing held on 7/15/99: Present: Ahn, Hunt, Chen, Shore, Mahayni, Mozersky

CC: NDA 20-986 (orig., 1 copy), HFD-510(Koller, Malozowski, Rhee, J.), HFD-850(Lesko), HFD-870(M. Chen, Fossler, Ahn), HFD-340(Vish), Central Document Room (Barbara Murphy)

7/15/99

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Study 022

TITLE OF TRIAL A randomised double-blind crossover trial to compare the plasma insulin profile of a single dose of insulin analogue X14 with that of Human Actrapid® in healthy volunteers.
INVESTIGATOR [ ]
TRIAL CENTRE
PUBLICATIONS None
TRIAL PERIOD 31 January 1995 to 12 July 1995 — **CLINICAL PHASE** Phase I
OBJECTIVES The primary objective was to compare the plasma profile of Human Insulin Analogue X14 (X14) with that of Actrapid® HM (ge) (Actrapid). The secondary objective was to compare the glucose profiles obtained with the two compounds.
METHODOLOGY Single-centre, randomised, double-blind 2-period crossover trial in which the subjects received either a single dose of X14 or Actrapid on each of two study days.
NUMBER OF SUBJECTS - 28 subjects were screened - 25 subjects were enrolled in the trial - 24 completed the trial Twenty-four (24) completed the trial as per protocol. In accordance with the protocol only 19 were included in the efficacy analysis of paired data. Twenty-five (25) were included in the safety analysis. One subject was withdrawn due to protocol violation.
DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION Healthy, non smoking, Caucasian males, aged 18 to 50 years, HbA_{1c} < 6.1%, fasting blood glucose < 6 mmol/l, body mass index < 30 kg/m^2 and willingness to give written informed consent.
TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER Human Insulin Analogue X14 (0.1 U/kgbw; injected s.c. into the anterior abdominal wall midway between the umbilicus and the anterior superior iliac spine, by use of a NovoPen® – ; batch number: 07794)
DURATION OF TREATMENT Two treatment days. Single dose.
REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER Actrapid® HM (ge) (0.1 IU/kgbw; injected s.c. into the anterior abdominal wall midway between the umbilicus and the anterior superior iliac spine, by use of a NovoPen® – batch number: 46162)
CRITERIA FOR EVALUATION - EFFICACY 24-points plasma insulin, plasma glucose and C-peptide profiles. **CRITERIA FOR EVALUATION - SAFETY**

General physical examination, haematology, biochemistry, and adverse events.

STATISTICAL METHODS

The primary endpoint $MRT_{(ins)}$ and the secondary endpoints $C_{max(ins)}$, $T_{max(ins)}$ and $AUC_{(ins)}$ were derived from plasma insulin profiles in a interval from 0 to 480 minutes. The secondary endpoints $AUC_{(BG)}$, $\Delta C_{min(BG)}$ (the maximum change from baseline to minimum concentration) and $T_{min(BG)}$ were derived from blood glucose profiles in a interval from 0 to 480 minutes. The primary endpoint $MRT_{(ins)}$ and the secondary endpoints $C_{max(ins)}$, $AUC_{(ins)}$ and $AUC_{(BG)}$ were logarithmically transformed and analysed by analysis of variance (ANOVA) with subject as a random effect and treatment condition as a fixed effect. A comparison of the two treatments with respect to $T_{max(ins)}$ for insulin and $T_{min(BG)}$ for blood glucose was done by evaluating the median difference in $T_{max(ins)}$ and $T_{min(BG)}$, respectively. The analysis was performed by Wilcoxon Signed Rank test. A non-parametric 95% confidence interval for the median difference was constructed. All tests were done as within subject comparisons. A significance level of 5% was used throughout the analyses.

EFFICACY RESULTS

The primary insulin endpoint $MRT_{(ins)}$ was statistically significantly lower for X14 than for Actrapid. The secondary insulin endpoints $AUC_{(ins)}$ and $C_{max(ins)}$ were significantly higher for X14 than for Actrapid, and $T_{max(ins)}$ was significantly lower. The secondary blood glucose endpoints showed no statistically significant difference in $AUC_{(BG)}$, whereas a significantly higher $\Delta C_{min(BG)}$ value and significantly lower $T_{min(BG)}$ value for X14 than for Actrapid was shown. All the endpoints were based on 19 subjects. Five of the 24 subjects who completed the trial, terminated the profiles on X14 after approximately one hour due to hypoglycaemia. These subjects were therefore not included in the efficacy analyses.

Primary Insulin Endpoints: Means (SD)

	$MRT_{(ins)}$ (min)
X14	149.2 (25.5)
Actrapid	216.8 (30.3)

Secondary Insulin Endpoints: Means (SD)

	$AUC_{(ins)}$ (mU/l x min)	$C_{max(ins)}$ (mU/l)	$T_{max(ins)}$ (min)
X14	6461.4 (1206.9)	40.9 (11.2)	51.8 (23.0)
Actrapid	4669.3 (835.9)	17.5 (4.3)	144.8 (93.3)

Secondary Blood Glucose Endpoints: Means (SD)

	$AUC_{(BG)}$ (mmol/l x min)	$\Delta C_{min(BG)}$ (mmol/l)	$T_{min(BG)}$ (min)
X14	445.1 (110.1)	2.1 (0.6)	93.9 (45.4)
Actrapid	435.8 (131.4)	1.4 (0.4)	226.4 (119.6)

SAFETY RESULTS

There were no serious adverse events, and no subjects were withdrawn due to adverse events. Twenty (20) subjects experienced a total of 45 non-serious adverse events. In addition, 3 non-serious adverse events were seen before any treatment. Twenty-six (26) adverse events occurred after treatment with X14 and 19 after treatment with Actrapid.

CONCLUSIONS

The results of this trial in healthy male volunteers showed that:

- X14 has a significantly faster absorption, reaches higher maximum insulin concentration, and returns to baseline faster than Actrapid
- the maximum change from baseline to minimum blood glucose concentration is significantly higher for X14 than for Actrapid, and the time to reach the minimum blood glucose concentration is reached faster for X14 than for Actrapid, which indicates that X14 has a faster onset of action. After approximately 3 hours the blood glucose levels were higher for X14 than for Actrapid, which shows that X14 has a shorter duration of action than Actrapid
- no safety concerns about X14 were raised by this trial

Ethics Committee approval and signed informed consent were obtained prior to starting the trial. The trial was conducted in accordance with the Helsinki Declaration and with Good Clinical Practice.

Synopsis for 044/UK

TITLE OF TRIAL A randomised, double-blind single centre two-way crossover trial testing the bioequivalence of two Human Insulin Analogue X14 products, produced by two different production methods, in healthy volunteers.
INVESTIGATOR
TRIAL CENTRE
PUBLICATIONS None

TRIAL PERIOD	**DEVELOPMENT PHASE**
26 September 1996 to 8 February 1997.	I

OBJECTIVES To test the bioequivalence of two Human Insulin Analogue X14 products, produced by two different production methods, in healthy males and females after subcutaneous (sc) administration.
METHODOLOGY The trial was a double-blind, randomised crossover design in which healthy subjects received a single dose, by sc administration, of two fast acting insulins produced by two different production methods. The washout period between doses was 4-10 days. The subjects were monitored for a 10-hour period after dosing whilst fasting. During this period, blood samples were collected over 30 timepoints for the determination of serum insulin, glucose and C-peptide.
NUMBER OF SUBJECTS - Twenty-five (25) subjects, 13 males and 12 females, were randomised and enrolled in the trial. - Twenty-four (24) subjects completed the trial and one was withdrawn due to an adverse events (loss of consciousness). - Twenty-five (25) subjects were included in the safety analysis and 23 subjects were included in the efficacy analysis.
DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION Healthy male subjects, 18-50 years of age, and healthy female subjects, 18-40 of age, with a body mass index of < 27 kg/m^2 and a fasting blood glucose ≤ 6 mmol/l. Written informed consent had to be obtained before inclusion of a subject in the trial.
TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER Human Insulin Analogue X14 new production method (X14 New Method) (0.06 U/kg BW) administered by sc injection into a skinfold perpendicular to the anterior abdominal wall, on a line between the umbilicus and the anterior superior iliac spine (50-100 mm lateral to the umbilicus); batch number: C96017; expiry date: 20 May 1998.
DURATION OF TREATMENT Single dose of each insulin product separated by a washout period of 4-10 days

REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

Human Insulin Analogue X14 old production method (X14 Old Method) (0.06 U/kg BW) administered by sc injection into a skinfold perpendicular to the anterior abdominal wall, on a line between the umbilicus and the anterior superior iliac spine (50-100 mm lateral to the umbilicus); batch number C96009; expiry date: 4 March 1998.

STATISTICAL METHODS

The statistical model included the fixed effects: treatment, sequence, visit, gender and the random subject (within gender and sequence) effect.

The endpoints $AUC_{ins(0-10)}$, $C_{max(ins)}$, $C_{min(bg)}$, and $AOC_{bg(0-10)}$ were logarithmically transformed (using the natural logarithm) before they were subject to analysis of variance.

For these endpoints, the mean difference on the log scale between the two formulations was estimated and the 90% confidence interval for the mean difference was calculated. The confidence intervals were then detransformed to give a 90% confidence interval for the ratio. The mean difference was also detransformed to give the geometric mean ratio.

The endpoints $t_{max(ins)}$ and $t_{min(bg)}$ were analysed non-parametrically using the Wilcoxon Signed Rank test on the paired differences. A 90% confidence interval of the median difference was constructed, where the median was the Hodges-Lehman estimator.

In order to declare bioequivalence the confidence interval for the primary endpoint had to be completely contained in the [redacted] interval.

EFFICACY RESULTS

The results of this trial confirmed that insulin from the two formulations of X14, X14 Old method and X14 New method is rapidly absorbed, with C_{max} occurring at approximately 1 hour and their duration of action is short (returning to baseline by 6 hours post-dose).

After administration of X14 New Method, one subject showed no response with respect to insulin levels and a further subject showed only a small increase in insulin levels compared to other subjects. These subjects also failed to show a normal response in serum glucose levels. It is likely that the subjects did not receive the full dose. The most likely reason for a failure in dosing is that the required airshot, to be made prior to dose, was not made completely.

When all subjects with evaluable efficacy data were included in the statistical analysis, bioequivalence between X14 Old Method and X14 New Method was not established based on the serum insulin primary endpoint, $AUC_{ins(0-10)}$. Analysis of the insulin secondary endpoint, $C_{max(ins)}$, including all subjects, supported this conclusion. However, when the two subjects who did not receive the full dose were excluded from the analysis of the primary endpoint, $AUC_{ins(0-10)}$ and the secondary endpoint $C_{max(ins)}$ the two formulations were shown to be bioequivalent.

The primary endpoint, $AUC_{ins(0-10)}$ is summarised in the table overleaf for each of the two treatments, and includes all evaluable subjects.

EFFICACY RESULTS -CONTINUED

	Insulin AUC(0-109(mU/l x h)	
	X14 Old Method	X14 New Method
N	23	23
Arithmetic Mean	43.524	40.301
Geometric Mean	42.203	36.633
Minimum		
Median	43.383	40.127
Maximum		
Std	11.396	13.675
CV (%)	26.184	33.933

The results of the test for bioequivalence between the two formulations of X14 for all evaluable subjects are presented in the table below:

X14 New Method versus X14 Old Method	Estimated Geometric Mean Ratio	Lower 90% C.I.	Upper 90% C.I.
AUC(0-10)	0.84	0.69	1.03

The analysis results of the primary endpoint excluding the subjects that did not receive the full dose, are shown in the table below:

X14 New Method versus X14 Old Method	Estimated Geometric Mean Ratio	Lower 90% C.I.	Upper 90% C.I.
AUC(0-10)	1.00	0.92	1.10

The conclusion of this trial was based on the analysis excluding the two subjects, i.e. that bioequivalence between X14 Old Method and X14 New Method was established based on the endpoints, $AUC_{ins(0-10)}$ and C_{max}.
There were no statistically significant differences between X14 Old and New Method for the insulin secondary endpoint, $t_{max(ins)}$, and glucose secondary endpoints, $AOC_{bg(0-10)}$, $C_{min(bg)}$ and $t_{min(bg)}$, when all subjects were included in the analysis.

There were no statistically significant differences between gender for any of the efficacy parameters.

SAFETY RESULTS

There were no serious adverse events reported during the trial. Of the 68 treatment-emergent adverse events reported during the trial, 57 were mild, 8 were moderate, 2 were severe and one was of unknown severity. One subject was withdrawn from the trial after administration of X14 New Method, due to adverse events including two severe events of loss of consciousness and tremor. These events were considered to be due to hypoglycaemia or a vasovagal attack. X14 Old Method and X14 New Method had similar adverse event profiles and the majority of events appeared to be due to hypoglycaemia symptoms. There were no clinically significant findings for clinical laboratory tests, vital signs, ECGs and physical examinations during the trial.

CONCLUSIONS

Based on the analysis of the primary end-point (serum insulin $AUC_{ins(0-10)}$) bioequivalence between X14 New Method and X14 Old Method was established when the two subjects that were likely not to have received the full dose of X14 New Method were excluded. This conclusion was supported by the analysis of the insulin secondary endpoint, $C_{max(ins)}$. However, when the analysis was performed including all evaluable subjects, bioequivalence was not established based on the endpoints, $AUC_{ins(0-10)}$ and $C_{max(ins)}$.

Bioequivalence between X14 Old and New Method was supported by the analysis of the insulin secondary endpoint, $t_{max(ins)}$, and glucose secondary endpoints, $AOC_{bg(0-10)}$, $C_{min(bg)}$ and $t_{min(bg)}$, when all subjects were included.

The incidence, severity and type of adverse events for X14 Old Method and X14 New Method was similar. The safety profile was similar between treatments and the majority of adverse events appeared to be related to hypoglycaemia.

There were no safety concerns with X14 Old Method and X14 New Method administration during the trial.

Ethics Committee approval and written informed consent were obtained prior to starting the trial. The trial was conducted in accordance with the Declaration of Helsinki and with Good Clinical Practice.

Synopsis for 045/UK

TITLE OF TRIAL A randomised, open labelled single centre two-way cross-over trial comparing the kinetics of two Insulin products, Novo Nordisk Insulin Analogue X14 and Lilly Insulin lispro, in healthy volunteers.
INVESTIGATOR [redacted]
TRIAL CENTRE [redacted]
PUBLICATIONS None

TRIAL PERIOD	**DEVELOPMENT PHASE**
18 September 1996 to 12 December 1996.	I

OBJECTIVES Comparison of the kinetics of two Insulin products, Novo Nordisk X14 and Lilly lispro (Humalog®), in healthy males after subcutaneous (sc) administration.
METHODOLOGY An open-labelled randomised cross-over design in which healthy subjects received a single dose of two fast acting insulin products separated by a washout period of 4-10 days.
NUMBER OF SUBJECTS - Twenty-seven (27) subjects were randomised and enrolled in the trial. - Nineteen (19) subjects completed the trial and eight were withdrawn; seven due to events considered related to hypoglycaemia and one due to an adverse event (vomiting) unrelated to hypoglycaemia. - Twenty-seven (27) subjects were included in the safety analysis and 19 subjects were included in the efficacy analysis.
DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION Healthy, non-smoking, male volunteers, 18-50 years of age with a body mass index of < 27 kg/m^2 and a fasting blood glucose ≤ 6 mmol/l. Written informed consent had to be obtained before inclusion of a volunteer in the study.
TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER Human Insulin Analogue X14 (0.08 U/kg BW) administered by subcutaneous (sc) injection into a skinfold perpendicular to the anterior abdominal wall, on a line between the umbilicus and the anterior superior iliac spine (50-100 mm lateral to the umbilicus) by use of a needle and syringe; batch number C96017.
DURATION OF TREATMENT Single dose of each insulin product separated by a washout period of 4-10 days.
REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER Insulin lispro (Humalog®) (0.08 U/kg BW) administered by subcutaneous (sc) injection into a skinfold perpendicular to the anterior abdominal wall, on a line between the umbilicus and the anterior superior iliac spine (50-100 mm lateral to the umbilicus) by use of a needle and syringe; batch number FF6F25F.

CRITERIA FOR EVALUATION - EFFICACY

The primary efficacy parameter was:

$AUC_{ins(0-10)}$, the area under the insulin concentration time curve from 0 to 10 hours.

The secondary efficacy parameters were:

$C_{max(ins)}$, the maximum insulin concentration in the interval 0 to 10 hours.

$t_{max(ins)}$, time to maximum insulin concentration in the interval 0 to 10 hours.

MRT_{ins}, mean residence time of insulin in the interval 0 to 10 hours.

$C_{min(bg)}$, the minimum glucose concentration in the interval 0 to 10 hours.

$t_{min(bg)}$, the time to minimum glucose concentration

$AOC_{bg(0-10)}$, the area below the baseline and above the glucose concentration time curve in the interval 0 to 10 hours.

CRITERIA FOR EVALUATION - SAFETY

Haematology, clinical chemistry, urinalysis, vital signs, physical examination, 12-lead ECG and adverse events.

STATISTICAL METHODS

The endpoints $AUC_{ins(0-10)}$, $C_{max(ins)}$, $C_{min(bg)}$, MRT_{ins} and $AOC_{bg(0-10)}$ for both treatments were logarithmically transformed (using the natural logarithm) before they were subject to analysis of variance.

For these endpoints, the mean difference on the log scale between the two formulations were estimated and the 90% confidence intervals for the mean differences were calculated. The confidence intervals were then detransformed to give a 90% confidence interval for the ratio. The mean difference was also detransformed to give the geometric mean ratio.

The endpoints $t_{max(ins)}$ and $t_{min(bg)}$ were analysed non-parametrically using a Wilcoxon Signed Rank test on the paired differences. A 90% confidence interval of the median difference was constructed, where the median was the Hodges-Lehman estimator.

In order to declare equivalence between X14 and Humalog® the confidence interval for the insulin primary endpoint, $AUC_{ins(0-10)}$ should be completely contained in the ———— interval.

EFFICACY RESULTS

Based on the analysis of the insulin primary endpoint, $AUC_{ins(0-10)}$, equivalence was established. This was supported by the analysis of the insulin secondary endpoint MRT_{ins} and the glucose secondary endpoints, $C_{min(bg)}$ and $AOC_{bg(0-10)}$. Furthermore, there was no statistically significant difference in the glucose secondary endpoint $t_{min(bg)}$.

For the insulin secondary endpoint $C_{max(ins)}$, the 90% confidence interval (77% to 95%) was just outside the specified ———— interval, and the results showed the Humalog® reached a significantly higher maximum insulin level ($C_{max(ins)}$) than X14 by approximately 14%. This finding was reflected in a statistical significant difference in the insulin primary endpoint $AUC_{ins(0-10)}$, which for X14 was 90% of that of Humalog® with a 90% confidence interval of 85% to 95%. The higher maximum insulin level for Humalog® was reached faster than for X14, however, this difference (5 minutes) was not clinically significant.

SAFETY RESULTS

There were no serious adverse events reported during the trial. Of the 71 treatment-emergent adverse events reported during the trial, 69 were mild and 2 were moderate in severity. X14 and Humalog® had similar adverse event profiles and the majority of events appeared to be related to hypoglycaemia. Eight subjects were withdrawn from the trial, seven due to hypoglycaemia or adverse events related to hypoglycaemia and one due to an adverse event unrelated to hypoglycaemia. All the subjects experiencing hypoglycaemia symptoms were withdrawn before or close to $t_{max(ins)}$, i.e. within one hour after dosing. All subjects withdrawn from the trial made a complete recovery.

There were no clinically significant findings for clinical laboratory tests, vital signs, ECGs and physical examinations during the trial.

CONCLUSIONS

- Based on the analysis of the insulin primary endpoint, $AUC_{ins(0-10)}$, equivalence between X14 and Humalog® was established. A statistically significant difference was also demonstrated for the insulin primary endpoint; $AUC_{ins(0-10)}$ for X14 being 90% of that for Humalog®.
- Equivalence was also demonstrated for the glucose secondary endpoints, $C_{min(bg)}$ and $AOC_{bg(0-10)}$, and for the secondary insulin endpoint MRT_{ins}. There was no statistically significant difference in the secondary glucose endpoint $t_{min(bg)}$, and no clinically relevant difference in the secondary insulin endpoint $t_{max(ins)}$ was shown.
- For the insulin secondary endpoint $C_{max(ins)}$, the 90% confidence interval (77% to 95%) was just outside the specified ——— , interval, and the results showed that Humalog® reached a significantly higher maximum insulin level ($C_{max(ins)}$) than X14 by approximately 14%.
- The safety profile was similar between treatments and the majority of adverse events appeared to be related to hypoglycaemia.
- There were no safety concerns about X14 and Humalog® raised during the trial.

Ethics Committee approval and written informed consent were obtained prior to starting the trial. The trial was conducted in accordance with the Declaration of Helsinki and with Good Clinical Practice.

Synopsis for 023/D

TITLE OF TRIAL A two period double-blind randomized crossover trial to compare the pharmacodynamic response to a single dose of Insulin Analogue X14 with that of Actrapid® HM (ge) in healthy volunteers during a euglycaemic clamp.
INVESTIGATOR Dr. Tim Heise, University of Düsseldorf, Postfach 10 10 07, 40001 Düsseldorf, Germany.
TRIAL CENTRE University of Düsseldorf, Postfach 10 10 07, 40001 Düsseldorf, Germany.
PUBLICATION Heinemann L, Kapitza C, Starke AAR, Heise T. Duration of Action of the Insulin Analogue B28Asp in Comparison to Regular Insulin. Diabetes 1996;45(2):509.
TRIAL PERIOD 3 February 1995 to 20 April 1995 — **CLINICAL PHASE** Phase I
OBJECTIVES To compare the pharmacokinetics of, and pharmacodynamic response to, a single dose of Human Insulin Analogue X14 (X14) with that of Actrapid® HM (ge) (Actrapid) in healthy male volunteers, by means of a euglycaemic clamp.
METHODOLOGY Single-centre, randomized, double-blind crossover trial in which the subjects received either a single dose of X14 or Actrapid on each of two study days during a euglycaemic clamp.
NUMBER OF SUBJECTS (PLANNED AND ANALYSED) - 26 subjects were screened - 24 subjects were enrolled - 24 completed the trial
DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION Healthy, non smoking, Caucasian males, aged 18 to 40 years, HbA_{1c} < 6.1%, fasting blood glucose < 6 mmol/l, body mass index < 27 kg/m^2, and willingness to give written informed consent.
TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER Human Insulin Analogue X14 (0.2 U/kgbw); injected s.c. perpendicular into a skinfold of the anterior abdominal wall, on a line between the umbilicus and the anterior superior iliac spine (50-100 mm lateral to the umbilicus), by use of a NovoPen® batch number: 07794.
DURATION OF TREATMENT Two treatment days, separated by one to two weeks.
REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER Actrapid® HM (ge) (0.2 IU/kgbw); injected s.c. perpendicular into a skinfold of the anterior abdominal wall, on a line between the umbilicus and the anterior superior iliac spine (50-100 mm lateral to the umbilicus), by use of a NovoPen® batch number: 46162.
CRITERIA FOR EVALUATION - EFFICACY Glucose infusion rate (GIR) profiles and 30-point serum insulin profiles. **CRITERIA FOR EVALUATION - SAFETY** General physical examination; clinical laboratory parameters (haematology and biochemistry); adverse events.

STATISTICAL METHODS

The primary endpoints GIR_{max}, $TGIR_{max}$, AUC_{GIR}, and $T_{AUC½}$ were derived from the GIR profiles in the interval from 0 to 600 minutes. Secondary endpoints C_{max}, T_{max}, AUC_{INS}, and MRT were derived from the insulin profiles in the interval from 0 to 600 minutes.

To compare the two treatments, the endpoints GIR_{max}, AUC_{GIR} and $T_{AUC½}$ MRT, C_{max}, and AUC_{INS} were subjected to an analysis of variance with subject as a random effect and treatment as a fixed effect. A comparison of treatment with respect to $TGIR_{max}$ and T_{max} was done by evaluating the median difference in $TGIR_{max}$ and T_{max}, by using the Wilcoxon Signed Rank Test. GIR_{max}, AUC_{GIR}, MRT, C_{max} and AUC_{INS} were logarithmically transformed before analysis in order to fulfil the assumptions for the analysis of variance. All tests were made as within subject comparisons and at a 5% significance level.

EFFICACY RESULTS

The primary glucose endpoint GIR_{max} was higher for X14 than Actrapid, although the difference was not statistically significant. The primary glucose endpoints AUC_{GIR}, $T_{AUC½}$ and $TGIR_{max}$ showed statistically significantly lower values for X14 than Actrapid. The secondary insulin endpoints C_{max} and AUC_{INS} were statistically significantly higher for X14 than Actrapid, whereas T_{max} and MRT were statistically significantly lower for X14. All the endpoints are based on N = 24.

Primary Glucose Endpoints: Means (SD)

	GIR_{max} (mg/(min x kg))	AUC_{GIR} (g/kg)	$T_{AUC½}$ (min)	$TGIR_{max}$ (min)
X14	13.1 (2.4)	3.4 (0.6)	193.8 (21.7)	133.8 (46.5)
Actrapid	12.1 (2.6)	3.7 (0.7)	235.9 (23.4)	180.8 (56.8)

Secondary Insulin Endpoints: Means (SD)

	AUC_{INS} (U/l x min)	C_{max} (mU/l)	MRT (min)	T_{max} (min)
X14	21.4 (2.1)	125.8 (31.8)	182.1 (16.0)	50.2 (19.0)
Actrapid	18.2 (1.9)	55.2 (8.5)	237.9 (21.7)	125.2 (67.5)

SAFETY RESULTS

There were no serious adverse events. Two mild adverse events were seen: hyperbilirubinemia and elevated aspartate amino transferase level (GOT).

CONCLUSION

The results of this trial in healthy male volunteers showed that:

- X14 has a significantly faster absorption and onset of action, reaches higher maximal insulin concentration, and has a shorter duration of action compared to Actrapid,
- no safety concerns about X14 are raised by this trial.

Ethics Committee approval and signed informed consent were obtained prior to starting the trial. The trial was conducted in accordance with the Helsinki Declaration and with Good Clinical Practice.

Synopsis for 026/USA

TITLE OF TRIAL A Six-period, Double-blind, Randomized, Cross-over Trial to Compare the Action Profile of Insulin Analogue (X-14) in Healthy Subjects Using Different Injection Sites during an Euglycemic Clamp, and to Compare X-14 Profiles with Profiles of Novolin® R
PRINCIPAL INVESTIGATOR [redacted]
TRIAL CENTER [redacted]
PUBLICATIONS None

TRIAL PERIOD	**CLINICAL PHASE**
16 March 1996 to 23 July 1996	I

OBJECTIVES To compare the pharmacokinetic profiles and the pharmacodynamic response to single doses of X-14 injected subcutaneously using three different injection sites in healthy subjects during an euglycemic clamp and to compare these profiles with profiles of Novolin®R injected at the same sites.
METHODOLOGY Single-centre, six-period, double-blind, randomized, crossover study. After screening, qualified healthy volunteers were randomized to single subcutaneous doses of X-14 or Novolin®R in deltoid, abdomen, or thigh on 6 separate days. Doses were separated by at least a one-week washout period.
NUMBER OF SUBJECTS Screened: 24; randomized: 20; completed: 18 Two subjects were withdrawn from the trial: One because of a positive drug screen, and one for personal reasons. Twenty subjects were included in the safety and efficacy analysis. Furthermore, an additional analysis on the 18 completed subjects was performed.
DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION Healthy men, 18 to 40 years of age, body mass index ≤ 27.0 kg/ m^2 and fasting plasma glucose < 108 mg/dL. The randomized subjects had a mean age of 31 years (range: 19-40) and a mean body mass index of 23.6 kg/ m^2.
TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER Insulin Analogue (X-14) Regular Insulin 100 U/ mL, Batch no. C95001, expiration date 11/1996 Dose: 0.2 U/kg body weight, by subcutaneous injection in abdomen, deltoid or thigh
REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER Novolin® R 100 U/ mL, Batch no. 56169, expiration date 12/1997 Dose: 0.2 U/kg body weight, by subcutaneous injection in abdomen, deltoid or thigh
DURATION OF TREATMENT Six single doses, separated by a minimum 1-week washout period. Subjects were sequestered for 24 hours.

CRITERIA FOR EVALUATION - Pharmacokinetic/Pharmacodynamic

Primary endpoints: Maximal glucose infusion rate (GIR_{max}), time of maximal glucose infusion rate $(T_{max})_{GIR}$, area under the curve $(AUC)_{glucose}$, and time of 50% glucose disposal ($T_{AUC½}$), derived from glucose infusion rate (0 to 600 min).

Secondary endpoints: Insulin mean residence time (MRT), maximal concentration $(C_{max})_{insulin}$, the time of maximal insulin concentration (T_{max}), and the area under the curve $(AUC)_{insulin}$, derived from serum insulin (0 to 600 min).

CRITERIA FOR EVALUATION - SAFETY

Safety assessments were pre- to post-study changes in hematology and biochemistry laboratory tests, physical examination, weight, vital signs, ECG, continuous electrocardiogram (ECG) monitoring, and adverse event (AE) reporting.

STATISTICAL METHODS

The null hypothesis, that the three injection sites were equal was tested against the alternative that at least two of the sites differed. Also, PHARMACODYNAMICS/PK parameters for X-14 insulin and Novolin® R were compared for each injection site. Mean differences between the three injection sites and between treatments for each of the three sites were estimated and 95% confidence intervals were constructed.

AUC_{GIR}(0-600 min), GIR_{max}, $T_{AUC½}$, AUC_{INS}, C_{max}, and MRT were log-transformed before analysis by ANOVA with injection site, treatment, period, as fixed effects and subject as a random effect. T_{max} was compared by non-parametric methods.

EFFICACY RESULTS

Glucose Infusion Rate: Ratios of mean AUC (0-600 mins) and GIR_{max} and the difference in T_{max} and $T_{AUC1/2}$ between the three injection sites were:

GIR Parameter Injection Sites, X-14	Ratio or Difference (min)	p-value	Lower 95% C.I.	Upper 95% C.I.
AUC (mg)				
Abdomen/Deltoid	0.899	0.025	0.821	0.986
Abdomen/Thigh	0.839	0.001	0.765	0.920
Deltoid/Thigh	0.933	0.130	0.851	1.022
GIR_{max} (mg/min)				
Abdomen/Deltoid	0.975	0.747	0.832	1.142
Abdomen/Thigh	0.988	0.882	0.842	1.160
Deltoid/Thigh	1.014	0.863	0.865	1.187
T_{max} (min)				
Abdomen - Deltoid	-22.840	0.196	---	---
Abdomen - Thigh	-41.310	0.060	---	---
Deltoid - Thigh	-12.920	0.541	---	---
$T_{AUC 1/2}$ (min)				
Abdomen - Deltoid	-27.6	0.113	---	---
Abdomen - Thigh	-34.3	0.000	---	---
Deltoid - Thigh	-5.9	0.258	---	---

Glucose infusion rate parameters for X-14 suggest a more rapid glucose lowering action for abdomen than deltoid or thigh injection sites. $T_{AUC 1/2}$ and T_{max} for abdomen were shorter than for deltoid or thigh. However, the total amount of glucose infused was slightly less for abdomen, as shown by the lower AUC. Differences in AUC and $T_{AUC 1/2}$ were statistically significant. Values for deltoid and thigh were similar. GIR_{max} was similar for all injection sites.

EFFICACY RESULTS -CONTINUED

GIR parameters between X-14 and Novolin® R were compared:

Parameter Injection Site	Ratio or Difference (min)	p-value	Lower 95% C.I.*	Upper 95% C.I.*
AUC (X-14/ Novolin® R)				
Deltoid	0.900	0.007	0.838	0.966
Abdomen	0.869	0.002	0.803	0.941
Thigh	0.985	0.743	0.893	1.087
GIR_{max} (X-14/ Novolin® R)				
Deltoid	1.177	0.036	1.012	1.369
Abdomen	1.154	0.006	1.050	1.270
Thigh	1.186	0.009	1.052	1.338
T_{max} (X-14 - Novolin® R)				
Deltoid	-98.700	0.000	---	---
Abdomen	-89.900	0.000	---	---
Thigh	-77.500	0.036	---	---
$T_{AUC\ 1/2}$ (X-14 - Novolin® R)				
Deltoid	-62.050	0.000	---	---
Abdomen	-69.755	0.000	---	---
Thigh	-48.522	0.001	---	---

*Confidence intervals were not calculated for non-parametric variables.

The T_{max} and $T_{AUC1/2}$ was shorter with X-14 than Novolin® R for each of the three injection sites. GIR_{max} was higher, but AUC was lower, with X-14 than Novolin® R. All differences, with the exception of AUC at the thigh injection site, were statistically significant. These results indicated a more rapid metabolic effect with X-14 compared with Novolin® R.

Insulin: Ratios of mean insulin AUC (0-600 min) and C_{max} (X-14/Novolin® R) and the difference between treatments for T_{max} and $T_{AUC\ 1/2}$ for the three injection sites were evaluated.

PK Parameter Injection Site	Ratio or Difference	p-value	Lower 95% C.I.*	Upper 95% C.I.*
AUC (X-14/ Novolin® R)				
Deltoid	1.228	0.002	1.096	1.376
Abdomen	1.183	0.002	1.079	1.296
Thigh	1.207	0.000	1.121	1.299
C_{max} (X-14/Novolin® R)				
Deltoid	2.115	0.000	1.683	2.658
Abdomen	1.998	0.000	1.652	2.416
Thigh	1.845	0.000	1.421	2.395
T_{max} (X-14 - Novolin® R)				
Deltoid	-25.000	0.004	---	---
Abdomen	-50.000	0.004	---	---
Thigh	-60.000	0.005	---	---
MRT (X-14/Novolin® R)				
Deltoid	0.713	0.000	0.652	0.778
Abdomen	0.707	0.000	0.663	0.753
Thigh	0.771	0.000	0.695	0.856

EFFICACY RESULTS -CONTINUED

Insulin AUC and C_{max} were greater, and T_{max} and MRT were less, with X-14 than with Novolin® R for each injection site. Differences in AUC, C_{max}, T_{max} and MRT for each site were significant. The differences are more pronounced when values were adjusted for endogenous insulin. These differences indicated faster and greater absorption of X-14 insulin than Novolin® R.

SAFETY RESULTS

There were no serious adverse events. One subject had a mild adverse event, soreness in the right arm at the IV line site, during treatment with Novolin® R. This event was considered unrelated to study drug. Mean changes in blood chemistry were within the reference range or not clinically significant. Decreases in hemoglobin, hematocrit and RBC are common with frequent blood sampling. Individual changes did not require treatment or clinical monitoring. Two subjects had a change in ECG during treatment with Novolin® R (deltoid) at one visit. Shifts in vital signs, weight, ECGs and physical exam findings were not clinically significant.

CONCLUSIONS

In the comparison of X-14 injection sites, the amount of glucose infused indicated that the onset of the glucose lowering action of X-14 did not differ between injection sites as assessed by similar GIR T_{max}. However, the duration of the glucose lowering action was up to 34 minutes shorter ($p = 0.000$) for the abdomen injections than for the deltoid or thigh injections, as assessed by GIR $T_{AUC\ ½}$. The total amount of glucose infused was significantly lower (10-14%) for the abdomen than for other sites. GIR and insulin parameters for X-14 in deltoid and thigh injections were clinically and statistically similar.

The pharmacodynamic data from this euglycemic clamp study showed a more rapid glucose lowering action of X-14 compared with Novolin® R. Times to maximal glucose infusion rate (T_{max}) and ½ AUC ($T_{AUC\ 1/2}$) were less and the maximal glucose infusion rate (GIR_{max}) was higher for X-14 than Novolin® R for each of the three injection sites. In addition, the difference in insulin pharmacokinetic properties was consistent with the pharmacodynamic difference in glucose infusion rate. X-14 was absorbed more rapidly, reached a higher maximal concentration and had a lower MRT.

Safety data for the 20 subjects indicated X -14 was well tolerated.

This trial was conducted according to the principles of Good Clinical Practice as outlined in 21 CFR Parts 50, 56, and 312 Subpart D.

Synopsis for 027/D

TITLE OF TRIAL A parallel, randomised, double-blind trial to investigate the intra-subject and inter-subject variations in action profiles after injection with Insulin X14 or Human Soluble Insulin during a euglycaemic clamp.
INVESTIGATOR [ ]
TRIAL CENTRE
PUBLICATIONS None
TRIAL PERIOD 6 November 1995 to 26 February 1996 — **DEVELOPMENT PHASE** Phase I
OBJECTIVES The primary objective was to investigate the intra-subject variation in the time action profile after four independent injections with either insulin X14 (X14) or Human Soluble Insulin (Actrapid), and secondly to investigate inter-subject variations.
METHODOLOGY The trial was designed as a single-centre, parallel-group, randomised, double-blind euglycaemic clamp trial with four study days. The subjects were randomised to either X14 or Actrapid. On each study day each healthy male volunteer received the treatment they were randomised to: A study day ended 10 hours after administration of the trial medication.
NUMBER OF SUBJECTS - 21 subjects were screened - 20 subjects were randomised - 18 subjects completed
DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION The main inclusion criteria were: healthy males between 18 and 40 years inclusive, with a body mass index (BMI) < 27 kg/ m^2, a HbA_{1c} < 6.1%, and a fasting blood glucose < 6 mmol/l. Subjects gave written informed consent before allocation to treatment or any trial procedure. The subjects randomised to X14 had a mean age of 25 years (SD 1.5), a BMI of 23 kg/m^2 (SD 2) a HbA_{1c} of 4.46 % (SD 0.49) and a fasting blood glucose of 4.56 mmol/l (SD 0.49). The subjects randomised to Actrapid had a mean age of 25 years (SD 1.5), a BMI of 23 kg/m^2 (SD 2) a HbA_{1c} of 4.54 % (SD 0.46) and a fasting blood glucose of 4.23 mmol/l (SD 0.98).
TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER Human Insulin analogue X14 (0.2 U/kg; batch number: C95001, injected perpendicularly into a skinfold of the left abdominal wall, on a line between the umbilicus and the anterior superior iliac spine (50-100 mm lateral of the umbilicus).
DURATION OF TREATMENT Four single doses. Each dose separated by 4 to 21 days.
REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER Actrapid® HM (ge) (0.2 IU/kg; batch number: 56169, injected as test product).

CRITERIA FOR EVALUATION - EFFICACY

Key assessment variables were glucose infusion rate (GIR) and serum concentrations of X14 and Actrapid.

CRITERIA FOR EVALUATION - SAFETY

General physical examination; vital signs; biochemistry; haematology; urinalysis; adverse events.

STATISTICAL METHODS

The primary endpoints were the following parameters derived from the smoothed GIR profiles: GIR_{max}, $TGIR_{max}$, $T_{AUC1/2}$, and AUC_{GIR}. The secondary endpoints were the following parameters derived from the serum insulin profiles: C_{max}, T_{max}, MRT (Mean Residence Time), and AUC_{INS}. All endpoints were derived in the time interval from drug administration (t=0) to last measured time point (t=600min.). Estimates of the intra- and inter-subject variations were obtained from analysis of variance. A standard F-test was applied to compare the variations of the endpoints between X14 and Actrapid. A significance level of 5% was used.

EFFICACY RESULTS

- $TGIR_{max}$ showed a statistically significantly lower intra-subject variability with X14 than with Actrapid.
- The MRT of the insulin profiles demonstrated a trend towards a lower inter-subject variation of X14 compared to Actrapid.
- C_{max} (inter-subject) and T_{max} (intra-subject) of the insulin profiles demonstrated a lower variability of X14 compared to Actrapid.
- Only the insulin profile derived endpoint, C_{max} demonstrated a higher variability (intra-subject) of X14 compared to Actrapid.
- This trial also confirmed that X14 exhibits a more rapid time action profile, a shorter duration of action and a more intense maximal effect compared to Actrapid as assessed by pharmacokinetic and pharmacodynamic measurements during euglycaemic clamping.

		X14/Actrapid Estimates	Ratio	P-Value
GIR derived endpoints:				
	Intra-Subject Variation			
	AUCgir	0.407/0.337	1.207	0.6266
	GIRmax	8.792/6.086	1.445	0.3423
	TAUC1/2	426.999/681.000	0.627	0.2258
	TGIRmax	2156.146/5067.444	0.425	0.0281
	Inter-Subject Variation			
	AUCgir	0.325/0.390	0.833	0.7857
	GIRmax	6.155/3.295	1.868	0.3912
	TAUC1/2	274.865/484.156	0.568	0.4163
	TGIRmax	0.000/863.295	0.000	.
Insulin derived endpoints:				
	Intra-Subject Variation			
	AUCins	3.24/2.79	1.161	0.7007
	Cmax	822.57/67.43	12.199	0.0000
	MRT	239.59/270.30	0.886	0.7522
	Tmax	1196.10/5344.33	0.224	0.0002
	Inter-Subject Variation			
	AUCins	1.79/1.84	0.972	0.9571
	Cmax	2.97/24.02	0.124	0.0051
	MRT	115.95/437.95	0.265	0.0640
	Tmax	88.04/0.00	.	.

SAFETY RESULTS The 6 adverse events which occurred during treatment with trial drug were equally distributed between the two treatment groups (3 events in each group). None of the events were severe or serious. No clinically relevant changes were seen in any safety parameter.
CONCLUSIONS • X14 displayed a lower intra-subject variability in the time to peak concentration and time to peak effect than Actrapid. • There were no other major differences in the intra- and inter-subject variability's in the pharmacokinetics and pharmacodynamics of X14 versus that of Actrapid. • The GIR and serum insulin mean profiles indicated that X14 had a faster onset of action, a shorter duration of action and reached higher glucose infusion rate and serum insulin concentration than Actrapid.
Ethics Committee approval and signed informed consent were obtained prior to starting the trial. The trial was conducted in accordance with the Helsinki Declaration and with Good Clinical Practice.

Synopsis for 039/UK

TITLE OF TRIAL A randomised, double-blind, single-centre crossover trial to demonstrate equivalence in the effect of Insulin Aspart and Human Soluble Insulin on ventricular repolarisation during hypoglycaemia in healthy volunteers.
INVESTIGATORS [redacted]
TRIAL SITE [redacted]
PUBLICATIONS None.

TRIAL PERIOD	DEVELOPMENT PHASE
18 July 1997 to 19 November 1997	Phase I

OBJECTIVES

The primary objective was to demonstrate equivalence in the effect of insulin aspart (IAsp) and human soluble insulin (HI) on ventricular repolarisation, as predicted by changes in the QTc interval of the electrocardiogram (ECG) during hypoglycaemia induced by hyperinsulinaemic blood glucose clamps.

The secondary objectives were to demonstrate equivalence in the effect of both insulins on ventricular repolarisation, as predicted by changes in QT dispersion (the difference between the longest and shortest QT interval) of the ECG during hypoglycaemia, and to demonstrate equivalence in the effect of both insulins on plasma concentrations of potassium, magnesium, calcium, catecholamines and glucagon during hypoglycaemia induced by hyperinsulinaemic blood glucose clamps.

METHODOLOGY

This was a randomised, double-blind, crossover study conducted at a single centre. The study took place over four visits. At Visit 1, healthy male subjects were screened for eligibility. Subjects were randomly assigned to receive either IAsp or HI at Visit 2, which took place 7 to 14 days after Visit 1. After a washout period of 28 to 56 days, subjects attended Visit 3 at which they crossed over to receive the alternative treatment. At Visits 2 and 3, each subject received an infusion of either IAsp or HI at 1.5 mU/kg/min for 120 minutes. Blood glucose was controlled by adjusting the rate of a 20% dextrose infusion. Blood glucose measurements were taken every five minutes. Blood glucose was to be maintained at 5 mmol/l for 30-60 minutes post start of infusion and then lowered to 2.5 mmol/l over the time period 90-120 minutes post start of infusion. Seven to 14 days later, subjects were required to attend Visit 4, the post-study visit. Prior to Visits 2 and 3, subjects were required to fast overnight (water was permitted).

NUMBER OF SUBJECTS

Twenty subjects were screened, of which 17 were randomised. All 17 completed the study.

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION

Healthy Caucasian male subjects aged between 18 and 40 years inclusive with a normal ECG including sinus rhythm and no signs of previous cardiac events, who had a body mass index (BMI) ≤ 27 kg/m^2, who were non-smokers for at least three months prior to screening, who were considered generally healthy upon completion of medical history, physical examination and

laboratory assessments and who had given signed informed consent.
TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER IAsp 100 U/ml provided in – ml cartridges and administered intravenously in a saline vehicle at an infusion rate of 1.5 mU/kg/min during the study procedure. Batch number: C96024.
DURATION OF TREATMENT Study drug was administered as a single dose on study day 2 (Visit 2) and study day 3 (Visit 3, 28 to 56 days after Visit 2).
REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER HI (Human Actrapid®) 100 IU/ml provided in 1.5 ml cartridges and administered intravenously in a saline vehicle at an infusion rate of 1.5 mIU/kg/min during the study procedure. Batch number: 56169.
CRITERIA FOR EVALUATION - EFFICACY Efficacy: Efficacy parameters were changes in QTc interval (measured continuously using a high resolution ECG system) and QT dispersion (measured using standard 12-lead ECGs) from normoglycaemia to hypoglycaemia following hyperinsulinaemic clamp. Also, blood samples were taken for measurement of glucose, potassium, magnesium, calcium, catecholamines, glucagon, insulin and C-peptide.
CRITERIA FOR EVALUATION - SAFETY Safety: Physical examination (review of head, ears, eyes, nose and throat, cardiovascular system, respiratory system, abdomen, musculoskeletal system, nervous system), vital signs (blood pressure/pulse, taken in a sitting position), 12-lead ECG, haematology, biochemistry and urine screens, 5 minute blood glucose monitoring and adverse event (AE) reporting were to be used to assess safety.
STATISTICAL METHODS The analyses were to be based on the bioequivalence approach with regard to hypotheses and tests. The tests were based on the two one-sampled t-tests approach, which is equivalent to determination of 90% confidence limits. The equivalence limits were set to ——— , corresponding to symmetrical limits on the log scale. The difference between normoglycaemia and hypoglycaemia for QTc was estimated and transformed by taking natural logarithms and analysed using an appropriate analysis of variance (ANOVA) model, fitted using PROC MIXED (SAS, Version 6.12). For the secondary endpoints, some of the values were negative. Therefore, the variables QT dispersion, potassium, magnesium, calcium, adrenaline, noradrenaline and glucagon were analysed by taking the natural logarithm of the mean during hypoglycaemia and performing the analysis using an analysis of covariance (ANCOVA) model, in the same way as the ANOVA model for QTc, but with the addition of the log transformed mean during normoglycaemia as a covariate. Additionally, QTc was also analysed in this way to be consistent with the secondary endpoints. These analyses were performed using PROC MIXED, with subject within sequence as a random effect, and sequence, period, treatment and the covariate fitted as fixed effects. The Wilcoxon Signed Rank Test was used to analyse the changes in laboratory measurements between Visits 1 and 4.
DEMOGRAPHY OF PATIENT POPULATION All subjects were Caucasians, 16 were non-smokers and one was an ex-smoker. Mean age was 28.0 years (SD, 6.4), mean weight was 78.3 kg (SD, 7.7), mean height was 182.5 cm (SD, 6.5) and mean body mass index was 23.5 kg/m^2 (SD, 1.9).
EFFICACY RESULTS Mean QTc intervals for subjects treated with IAsp, were 401.2 ms (SD, 23.4) and 450.6 ms (SD, 28.4) in normoglycaemia and hypoglycaemia, respectively, and for subjects treated with HI, were 396.4 ms (SD, 22.3) and 447.8 ms (SD, 25.4), respectively. The mean difference between QTc

intervals (hypoglycaemia minus normoglycaemia) was 49.4 ms for IAsp and 51.4 ms for HI. These differences gave an estimated ratio for IAsp versus HI of 91.2% (90% CI, 74.8, 111.1). Equivalence of treatments was defined as having occurred if the 90% CI lay entirely within the limits of ——— Thus, the treatments did not fulfil the equivalence criteria. However, non-inferiority was fulfilled as QTc with IAsp was shorter than with HI.

Equivalence in the effects of the two treatments was found in QT dispersion (90% CI, 81.6, 98.2) and plasma concentrations of potassium (90% CI, 98.1, 101.8), magnesium (90% CI, 98.5, 101.7), calcium (90% CI, 98.4, 100.8), noradrenaline (90% CI, 90.7, 108.5) and glucagon (90% CI, 92.9, 101.3). Equivalence of treatment effects was not found for plasma concentrations of adrenaline (90% CI, 96.0, 139.6). However, there was no significant difference between treatments.

Clearance was similar for the two forms of insulin (1.2 L/h/kg).

SAFETY RESULTS

There were two on-treatment AEs, which both occurred in subjects being treated with IAsp and were both in the central and peripheral nervous system body system. Subject 11 experienced moderate headache which resolved after 16 hours. This was judged to be a hypoglycaemic event and was classified as probably related to study drug. Subject 17 experienced a moderate migraine 24 hours after the study day which lasted 3 hours. It was unknown whether this was a hypoglycaemic event, but the Investigator commented that the subject had not experienced a migraine since being at school, 3 to 4 years previously. The migraine started with visual disturbance, then he developed headache. The Investigator judged that the event was possibly related to the study drug.

There were no SAEs and no withdrawals in the study.

There were no clinically significant changes in clinical laboratory parameters or vital signs (blood pressure, pulse rate, body weight). All subjects were normal at physical examination at Visits 1 and 4.

CONCLUSIONS

The two treatments were not equivalent in their effects on QTc during hypoglycaemia, according to the predetermined definition. However, the upper CI limit was below the —— This was further supported by an ANCOVA analysis which showed equivalence in QTc.

However, they were equivalent with regard to the effects on QT dispersion and the plasma concentrations of potassium, magnesium, calcium, noradrenaline and glucagon. Equivalence was not obtained for plasma adrenaline, but there was no statistically significant difference between the treatments.

Clearance of the two forms of insulin was similar.

Two subjects had AEs, in the central and peripheral nervous system, which were judged as related to IAsp treatment.

Ethics Committee approval and signed informed consent were obtained prior to starting the trial. The trial was conducted in accordance with the Helsinki Declaration and with Good Clinical Practice.

Synopsis for 024/UK

TITLE OF TRIAL

A randomised double blind three way crossover trial to compare the effects on postprandial glycaemic excursion of Insulin Analogue X14 given immediately before a test meal, with Human Actrapid® given immediately before or thirty minutes before a test meal in type 1 diabetics.

INVESTIGATOR

[ ]

TRIAL CENTRE

[ ]

PUBLICATIONS

None

TRIAL PERIOD	**CLINICAL PHASE**
8 May 1995 to 5 February 1996	Phase I

OBJECTIVES

The objective was to compare the postprandial plasma glucose profile of Insulin Analogue X14 (X14), when given at time t=0 minutes to the postprandial plasma glucose profile of Actrapid, when given at time t=0 minutes and t=-30 minutes in relation to a standard meal.

METHODOLOGY

Single-centre, double-blind, double-dummy, randomised, three-way crossover trial, in which the subjects received either a single dose of X14 at time t=0, or Actrapid at time t=-30 ($Actrapid_{t=-30}$), or Actrapid at time t=0 ($Actrapid_{t=0}$) in relation to a test meal (a standard breakfast), on each of three study days. Actrapid was given overnight via a euglycaemic clamp for blood glucose control and to achieve a pre-dose blood glucose between 5-8 mmol/l. Insulin and glucose were measured until six hours post dose.

NUMBER OF SUBJECTS (PLANNED AND ANALYSED)

- 37 subjects were screened
- 24 subjects were enrolled in the trial
- 22 subjects completed the trial; two were withdrawn: one due to adverse events (cough and cold: assessed not related to trial drug) and one for personal reasons.
  Twenty-four subjects were included in the safety analysis and 22 subjects were included in the efficacy analysis.

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION

Type 1 diabetic male subjects, with diabetes for at least 2 years, aged 18 to 50 years, $HbA_{1c} < 9\%$, body mass index $< 30\ kg/m^2$, meal stimulated C-peptide (1-3 hour postprandial) < 0.1 nmol/l, concomitant plasma glucose > 7 mmol/l, and no severe late diabetic complications. The 24 enrolled subjects had a mean age of 33.18 years (SD=8.63), a mean duration of diabetes of 11.36 years (SD=7.07), a mean BMI of 25.08 (kg/m/m) (SD=2.34), and a mean HbA_{1c} of 7.58% (SD=1.12).

TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

Human Insulin Analogue X14 (0.15 U/kgbw; injected s.c. using a NovoPen® — mid-way between the umbilicus and the anterior superior iliac spine alternating the left and right sides; batch number: 07794).

DURATION OF TREATMENT

Three treatment days comprising single administration of X14 or Actrapid. To maintain blindness all subjects received two injections. The first at time t= -30 minutes and the second at time t=0 minutes according to the following schedule:

- Placebo (t=-30) / X14 (t=0) referred to as treatment X14
- Placebo (t=-30) / Actrapid (t=0) referred to as treatment Actrapid$_{t=0}$
- Actrapid (t=-30) / Placebo (t=0) referred to as treatment Actrapid$_{t=-30}$

REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

Actrapid® HM (ge) (0.15 U/kgbw; injected s.c. using a NovoPen® — mid-way between the umbilicus and the anterior superior iliac spine alternating the left and right sides; batch number: 46164).

Placebo (injected s.c. using a NovoPen® — mid-way between the umbilicus and the anterior superior iliac spine alternating the left and right sides,; batch number: 07894).

CRITERIA FOR EVALUATION - EFFICACY

Piasma glucose profiles (from 0 to 240 minutes): the total excursion of blood glucose concentration ($EXC_{(BG)}$), maximum blood glucose concentration ($C_{max(BG)}$), time to maximum blood glucose concentration ($T_{max(BG)}$), and minimum blood glucose concentration ($C_{min(BG)}$), during the time interval from $T_{max(BG)}$ to time for last measurement.

Insulin profiles (from 0 to 360 minutes for X14 and Actrapid$_{t=0}$ and from -30 to 330 minutes for Actrapid$_{t=-30}$): Mean Residence Time ($MRT_{(ins)}$), maximum insulin concentration ($C_{max(ins)}$), time to maximum insulin concentration ($T_{max(ins)}$), and area under the insulin curve ($AUC_{(ins)}$)

CRITERIA FOR EVALUATION - SAFETY

Physical examination, haematology, biochemistry, urine screen, allergic reaction at the injection site, and adverse events.

STATISTICAL METHODS

All endpoints, except T_{max} (for both insulin and glucose), were logarithmically transformed prior to analysis, and analysed by ANOVA with subject as a random effect and treatment as a fixed effect. The analysis of T_{max} was based on pairwise treatment comparisons (X14/Actrapid$_{t=0}$ and X14/Actrapid$_{t=-30}$) using Wilcoxon signed rank test. A non-parametric 95% confidence interval for the median for each treatment was constructed. All tests were performed within subjects at the 5% significance level. Safety laboratory values were analysed for significant changes (on a 5% level) from pre- to post-trial visit by use of Wilcoxon Signed Rank test.

EFFICACY RESULTS

The primary blood glucose endpoint $EXC_{(BG)}$ was significantly lower for X14 than for both Actrapid treatments. The secondary blood glucose endpoint $C_{max(BG)}$ was significantly lower for X14 than for Actrapid$_{t=0}$, but not significantly different from Actrapid$_{t=-30}$. There was no statistically significant differences in $C_{min(BG)}$ and $T_{max(BG)}$ between X14 and Actrapid treatments. The secondary insulin endpoints $AUC_{(ins)}$ and $C_{max(ins)}$ were significantly higher for X14 than for both Actrapid treatments. $MRT_{(ins)}$ and $T_{max(ins)}$ were significantly lower for X14 than for Actrapid$_{t=0}$. All endpoints are based on 22 subjects.

EFFICACY RESULTS -CONTINUED:

Primary blood glucose endpoint: Means (SD)

	$EXC_{(BG)}$ (mmol/l x min)
$X14_{(0-240min)}$	891.20 (521.44)
$Actrapid_{t=0(0-240min)}$	1311.36 (511.66)
$Actrapid_{t=-30(0-240min)}$	1105.82 (571.35)

Secondary blood glucose endpoints: Means (SD)

	$C_{max(BG)}$ (mmol/l)	$C_{min(BG)}$ (mmol/l)	$T_{max(BG)}$ (min)
$X14_{(0-240min)}$	13.49 (3.49)	9.36 (5.45)	99.77 (83.07)
$Actrapid_{t=0(0-240min)}$	16.37 (3.37)	10.03 (3.53)	102.73 (49.90)
$Actrapid_{t=-30(0-240min)}$	14.45 (3.47)	9.10 (2.61)	121.14 (44.85)

Secondary insulin endpoints: Means (SD)

	$AUC_{(ins)}$ (mU/l·min)	$MRT_{(ins)}$ (min)
$X14_{(0-360min)}$	11836.07 (6599.76)	121.80 (17.56)
$Actrapid_{t=0(0-360min)}$	8820.64 (4932.83)	162.44 (18.76)
$Actrapid_{t=-30(-30-}$	9427.75 (4952.84)	122.55 (15.65)

	$C_{max(ins)}$ (mU/l)	$T_{max(ins)}$ (min)
$X14_{(0-360min)}$	82.09 (42.83)	39.09(17.16)
$Actrapid_{t=0(0-360min)}$	35.86 (20.13)	100.68(44.86)
$Actrapid_{t=-30(-30-}$	39.89 (21.87)	55.23 61.79)

SAFETY RESULTS

There were no serious adverse events. One subject (subject 13) was withdrawn from the trial due to adverse events (cough and cold). Twenty (20) subjects experienced a total of 72 non-serious adverse events. In addition, 3 non-serious adverse events were seen before any treatment. Thirty (30) adverse events occurred following treatment with X14 (13 assessed as related to trial drug), 25 following treatment with $Actrapid_{t=0}$ (11 assessed as related to trial drug), and 17 following treatment with $Actrapid_{t=-30}$ (10 assessed as related to trial drug). The most frequent adverse events were hypoglycaemia and headache.

CONCLUSIONS

The results of this trial in type 1 diabetic subjects showed that:

- X14 shows an improved postprandial blood glucose control compared with Actrapid given 30 minutes or 0 minutes prior to a breakfast
- the maximum blood glucose concentration was lower for X14 than for $Actrapid_{t=0}$, which is consistent with a higher maximum insulin concentration for X14 than for $Actrapid_{t=0}$
- X14 reaches higher maximum insulin concentration than both Actrapid treatments, and the maximum insulin concentration is reached faster for X14 than for Actrapid. Furthermore, X14 shows a shorter duration of action than $Actrapid_{t=0}$. The area under the insulin curve is significantly higher for X14 than for both Actrapid treatments
- there is no marked difference in numbers of drug related adverse events between X14, $Actrapid_{t=0}$, and $Actrapid_{t=-30}$
- no safety concerns about X14 were raised by this trial.

Ethics Committee approval and signed informed consent were obtained prior to starting the trial.

The trial was conducted in accordance with the Helsinki Declaration and Good Clinical Practice.

APPEARS THIS WAY
ON ORIGINAL

Synopsis for 043/DK

TITLE OF TRIAL A single center randomised, double-blind cross-over study on the pharmacokinetics of insulin aspart and soluble human insulin in pediatric type 1 diabetic subjects.
INVESTIGATOR
TRIAL CENTRE
PUBLICATIONS None

TRIAL PERIOD	**DEVELOPMENT PHASE**
25 August 1997 to 1 December 1997.	I

OBJECTIVES Primary - to compare the pharmacokinetics of insulin aspart with human insulin in pediatric type 1diabetic subjects to verify that the pharmacokinetic differences observed in adults also apply to children Secondary - to compare the post prandial serum glucose profiles of insulin aspart with those of soluble human insulin in pediatric subjects - to study the short-term safety of insulin aspart in pediatric subjects
METHODOLOGY A randomised double-blind two period cross-over design in which pediatric type 1 subjects received a single dose of two fast acting insulin products immediately prior to a breakfast, separated by a washout period of at least 3 days.
NUMBER OF SUBJECTS - 18 subjects (9 subjects aged 6 to 12 years and 9 subjects aged 13 to 17 years) were randomised and enrolled in the trial. - 18 subjects completed the trial and none were withdrawn. 18 subjects were included in the safety and efficacy analysis.
DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION Male and female subjects with type 1 diabetes and on insulin treatment for at least 24 months, 6 - 17 years of age and HbA1c $\leq$ 11%. Written informed consent had to be obtained before inclusion of a subject in the study.
TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER Insulin aspart (0.15 U/kg BW) administered by subcutaneous (sc) injection into a skinfold perpendicular to the anterior abdominal wall, on a line between the umbilicus and the anterior superior iliac spine by use of a NovoPen®; batch number C96024.
REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER Human insulin (0.15 IU/kg BW) administered by subcutaneous (sc) injection into a skinfold perpendicular to the anterior abdominal wall, on a line between the umbilicus and the anterior superior iliac spine by use of a NovoPen®; batch number 66125.
DURATION OF TREATMENT Single dose of each insulin product separated by a washout period of at least 3 days.

CRITERIA FOR EVALUATION – EFFICACY

- The primary efficacy parameter was:
  Maximum serum insulin concentration [$C_{max(ins)}$]
- The secondary efficacy parameters were:
  Time to maximum serum insulin concentration [$t_{max(ins)}$]
  Area under the concentration time curve of serum insulin [$AUC_{0\text{-}5\ h(ins)}$]
  Total baseline corrected excursion of serum glucose [$EXC_{0\text{-}4\ h(glu)}$ and $EXC_{0\text{-}5\ h(glu)}$]
  Minimum concentration of serum glucose [$C_{min(glu)}$]
  Time to minimum serum glucose [$t_{min(glu)}$]
  Maximum concentration of serum glucose [$C_{max(glu)}$]
  Time to maximum serum glucose [$t_{max(glu)}$]
- The exploratory efficacy parameters were:
  Area under the concentration time curve of serum insulin from time zero to infinity [$AUC_{0\text{-}\infty(ins)}$]
  Relative bioavailability of insulin aspart/human insulin for $AUC_{0\text{-}\infty(ins)}$ [F(AUC)]
  Elimination rate constant of serum insulin[$\lambda_{z(ins)}$]
  Elimination half-life of serum insulin [$t_{1/2(ins)}$]
  Mean residence time of serum insulin [$MRT_{(ins)}$]
  Minimum concentration of serum glucose [$C_{min(glu)}$] adjusted for baseline
  Time to minimum serum glucose [$t_{min(glu)}$] adjusted for baseline

CRITERIA FOR EVALUATION – SAFETY

Physical examination, vital signs, haematology and biochemistry laboratory analyses and adverse events reporting.

STATISTICAL METHODS

The secondary endpoints $AUC_{0\text{-}5\ h(ins)}$, $AUC_{0\text{-}\infty(ins)}$, $EXC_{0\text{-}4\ h(glu)}$ and $EXC_{0\text{-}5\ h(glu)}$ were calculated by the trapezoidal method. The primary and secondary endpoints $C_{max(ins)}$, $AUC_{0\text{-}5\ h(ins)}$, $AUC_{0\text{-}\infty(ins)}$, $EXC_{0\text{-}4\ h(glu)}$, $EXC_{0\text{-}5\ h(glu)}$ and $C_{min(glu)}$ were logarithmically transformed (using the natural logarithm) before they were subject to analysis of variance. The statistical model included factors accounting for subject as a random effect, treatment as a fixed effect and age group as a fixed effect. A 95% confidence interval of the mean ratio between treatments was constructed.
The endpoints $t_{max(ins)}$ and $t_{min(glu)}$ were analysed non-parametrically using the Wilcoxon Signed Rank test on the paired differences. A 95% confidence interval of the median difference between treatments was constructed.

EFFICACY RESULTS

C-peptide levels in all subjects were low which is typical of type 1 diabetics. No adjustment to the insulin concentrations was considered necessary to account for endogenous insulin.
The results of this trial confirmed that insulin aspart is rapidly absorbed in pediatric diabetic subjects, with $C_{max(ins)}$ occurring at approximately 45 minutes post-dose, and its duration of action is short (almost returning to baseline by 5 hours post-dose).
In this trial, statistically significant differences between insulin aspart and human insulin were found for the insulin primary endpoint, $C_{max(ins)}$ and the insulin secondary endpoints, $AUC_{0\text{-}5\ h(ins)}$ and $t_{max(ins)}$. For the two age groups, the maximum insulin levels were approximately twice as high for insulin aspart compared to human insulin and $C_{max(ins)}$ was reached twice as early. The extent of absorption over the first 5 hours, as determined by $AUC_{0\text{-}5\ h(ins)}$, was approximately 36% higher for insulin aspart than for human insulin. These findings are consistent with the findings of previous studies in healthy adult volunteers and in type 1 and 2 diabetic adult subjects in which a more than twice as fast absorption and about twice as high peak concentration of insulin aspart was demonstrated.

EFFICACY RESULTS - continued

The analysis results of the primary endpoint are shown in the table below:

Age group	Insulin aspart versus human insulin	Estimated Geometric Mean Ratio	P-value	df	Lower 95% C.I.	Upper 95% C.I.
6-12 years	Insulin Cmax	2.04	<0.001	16	1.68	2.48
13-17 years	Insulin Cmax	2.21	<0.001	16	1.82	2.69
All	Insulin Cmax	2.13	<0.001	16	1.85	2.44

There were no statistically significant treatment differences between insulin aspart and human insulin for the glucose secondary endpoints, $C_{max(glu)}$, $C_{min(glu)}$, $EXC_{0\text{-}4\ h(glu)}$, $EXC_{0\text{-}5\ h(glu)}$, $t_{max(glu)}$ and $t_{min(glu)}$. Previous studies in type 1 and 2 diabetic adult subjects demonstrated that insulin aspart gave a better post prandial glycaemic control for the first 4 hours following a meal. In the present trial, the excursion of glucose levels from baseline over this period was 22% lower for insulin aspart compared to human insulin, however this difference was not statistically significant.

SAFETY RESULTS

There were no serious adverse events reported during the trial. Of the 8 treatment-emergent adverse events reported during the trial, 6 were mild and 2 were moderate. The incidence of adverse events for insulin aspart and human insulin was similar. Only one adverse event for each treatment was considered to be possibly or probably drug related which was hypoglycaemia reported by one subject on two occasions, i.e. after the administration of insulin aspart and human insulin. The severity of hypoglycaemia was mild on each occasion and the symptoms were acceptable enough for the subject to continue with all study procedures.
There were no clinically significant findings for clinical laboratory tests, vital signs and physical examinations during the trial.

CONCLUSIONS

- Based on the primary insulin endpoint, $C_{max(ins)}$, and the secondary endpoints, $AUC_{0\text{-}5\ h(ins)}$ and $t_{max(ins)}$, the pharmacokinetic differences between insulin aspart and human insulin in pediatric type 1 diabetic subjects were similar to those observed in adults. The maximum insulin levels were approximately twice as high and were reached twice as fast for insulin aspart compared to human insulin and $AUC_{0\text{-}5\ h(ins)}$ for insulin aspart was approximately 36% higher.
- The post prandial glycaemic control tended to be improved with insulin aspart by approximately 22% compared to human insulin for the first 4 hours following breakfast. However, there were no statistically significant differences between insulin aspart and human insulin for the glucose secondary endpoints, $C_{max(glu)}$, $C_{min(glu)}$, $EXC_{0\text{-}4\ h(glu)}$, $EXC_{0\text{-}5\ h(glu)}$, $t_{max(glu)}$ and $t_{min(glu)}$.
- The incidence of adverse events for insulin aspart and human insulin was similar. One incidence of hypoglycaemia was reported by one subject after the administration of each treatment.
- There were no safety concerns about insulin aspart and human insulin raised during the trial.

Ethics Committee approval and written informed consent were obtained prior to starting the trial. The trial was conducted in accordance with the Declaration of Helsinki and with Good Clinical Practice.

Synopsis for 030/DK/N

TITLE OF TRIAL A randomised two-centre double-blind three-way crossover trial to compare the effects on post prandial glycaemic excursions of Insulin Analogue X14 (Insulin Aspart) given immediately before a test meal, with Human Actrapid® given immediately before or thirty minutes before a test meal in insulin treated Type 2 diabetic subjects
INVESTIGATORS [ ]
TRIAL CENTRES [ ]
PUBLICATIONS None

TRIAL PERIOD	DEVELOPMENT PHASE
16 April 1996 to 16 January 1997	II

OBJECTIVES

The objective of the trial was to compare the postprandial serum glucose profile of insulin aspart (IAsp), when given at time t = 0 minutes to the postprandial serum glucose profile of Human Actrapid®, when given at time t = 0 and t = -30 minutes in relation to a test meal in subjects with insulin treated Type 2 diabetes.

METHODOLOGY

A randomised two-centre, double-blind, three-period crossover trial, in which the subjects attended one pre-study visit, three study days (each separated by 1 to 3 weeks), and one post-study visit. On each study day each subject received two injections, one 30 minutes before and one immediately before a test meal, using a double dummy technique. The first injection contained either placebo or Human Actrapid® (0.15 IU/kg) and the second either IAsp (X14) (0.15 U/kg), Human Actrapid® (0.15 IU/kg), or placebo. Accordingly, the following 3 combinations of injections were given (in random order, one on each study Day):

- placebo $_{t=-30}$ and IAsp $_{t=0}$
- placebo $_{t=-30}$ and Actrapid $_{t=0}$ (Act_0)
- Actrapid $_{t=-30}$ (Act_{-30}) $_{and}$ placebo $_{t=0}$

Subjects were subjected to a euglycaemic clamp during the night before the trial product injections. Insulin, glucose and C-peptide were measured until six hours post-dose.

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ON ORIGINAL

NUMBER OF SUBJECTS

- a total of 24 subjects were planned to be randomised
- subject disposition is tabulated below

Subjects	Total Number
Screened	49
Randomized	25
Completed	23
Withdrawn	2
Withdrawn: Adverse Event	0
Withdrawn: Other	2

- subject no. 3 was withdrawn before receiving any trial products, due to hyperglycaemia outside the 5 to 8 mmol/l target range on Study Day 1
- subject no. 12 withdrew consent to participate in the trial before dosing (Act_0) on Study Day 3

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION

Male and female subjects with Type 2 diabetes, males and females, aged 40 to 75 years inclusive. Type 2 diabetes for at least 15 months; insulin treated for at least 3 months, but not within first year of diagnosis. Glucagon stimulated C-peptide ≥ 0.32 nmol/l, BMI ≤ 35kg/m2 and HbA_{1c} < 10 %. No severe late complications of diabetes.

TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

Insulin Aspart 100 U/ml, – ml cartridges
Injections of single doses of 0.15 IU/kg s.c. Batch no.: C95001 (both centres)

DURATION OF TREATMENT

Single-dose injection on each of three study days. Each study day separated by 1 to 3 weeks.

REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

Human Actrapid® 100 IU/ml, 1.5 ml cartridges
Placebo (Test-medium m-Cresol), – ml cartridges
Injections of single doses of 0.15 IU/kg s.c. Batch no.: 56169 (both centres)

CRITERIA FOR EVALUATION - EFFICACY

From the serum glucose profiles, derived in the interval t = 0 to 360 min:

The total excursion of serum glucose concentration ($EXC_{(glu)}$), maximum serum glucose concentration ($C_{max(glu)}$), time to maximum serum glucose concentration ($t_{max(glu)}$), minimum serum glucose concentration ($C_{min(glu)}$) in the interval from $t_{max(glu)}$ to t = 360 min.

CRITERIA FOR EVALUATION - SAFETY

Physical examination, haematology, biochemistry, urine screen, reaction at injection site, and adverse events.

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ON ORIGINAL

STATISTICAL METHODS

All endpoints, with the exception of t_{max}, were log-transformed prior to analysis of variance (ANOVA), with subject as a random effect and treatment as a fixed effect. Treatment comparison was presented by an estimated mean, a p-value, degrees of freedom and a 95% confidence interval. A comparison of the treatments with respect to t_{max} was done using the non-parametric Friedmann test. The Friedman test was followed by pair-wise comparison of the treatments (IAsp/Act$_0$ and IAsp/Act$_{-30}$) using the Wilcoxon Signed Rank Test. A non-parametric 95% confidence interval for the median for each treatment comparison was constructed. All tests were performed within subjects at the 5% significance level.

All safety data was listed and summarised to illustrate changes from pre- to post-trial visit. Laboratory data (haematology and biochemistry parameters) were summarised, and analysed for significant changes from pre- to post-trial by use of the Wilcoxon signed rank test.

DEMOGRAPHICS OF SUBJECT POPULATION

All subjects were European; 14 males and 11 females. They had a mean age of 59.7 (43.0-71.0) years, a mean BMI of 28.3 (21.9-35.0) kg/m^2, a baseline HbA_{1c} of 8.5 [illegible] %, a mean glucagon stimulated C-peptide value of 0.6 [illegible] nmol/l, and a mean duration of diabetes of 12.5 [illegible] years.

PHARMACODYNAMIC RESULTS

Primary Glucose Endpoint

The primary glucose endpoint, $EXC_{(glu)}$ 0-360 min, was statistically significantly smaller for IAsp compared to Act$_0$, indicating that the postprandial glucose control was improved with IAsp compared to Act$_0$. No difference could be demonstrated when comparing IAsp to Act$_{-30}$.

Summary and Analysis of Primary Glucose Endpoint

	EXC (mmol/l * min)		
	X14	Act	Act_30
N	22	22	22
Mean	899.4	1101.5	867.9
Median	661.9	972.0	791.0
Std	608.8	497.0	374.2
Min	[illegible]	[illegible]	[illegible]
Max	[illegible]	[illegible]	[illegible]

Analysis Results

	X14 vs. Act	X14 vs. Act_30
Estimated Ratio	0.739	0.916
P-value	0.010	0.436
Degrees of Freedom	42	42
95 % Confidence Interval	[0.590 ; 0.926]	[0.731 ; 1.148]

Secondary Glucose Endpoints: $C_{max(glu)}$, $t_{max(glu)}$, $C_{min(glu)}$ (t max(glu) to t = 360min)

$C_{max(glu)}$ was statistically significantly lower for IAsp compared to Act$_0$ but not compared to Act$_{-30}$. No statistically significant difference in $C_{min(glu)}$ or $t_{max(glu)}$ was observed when comparing IAsp to either of the Actrapid treatments. Note that C_{min} was derived in the interval t_{max} to $t_{=360min}$.

PHARMACOKINETIC RESULTS

- due to the trial design, the standard methods for estimating the trial product insulin could not be applied and the planned pharmacokinetic endpoints could not be analysed.
- an explorative non-comparative analysis of IAsp, using a new specific assay confirmed that IAsp was absorbed. Mean t_{max} was 75.5 (± 41.7)min.

SAFETY RESULTS

- no serious adverse events or adverse event withdrawals were reported
- 24 subjects experienced a total of 18 adverse events, all classified as mild or moderate. Of 6 adverse events experienced following IAsp, 3 were assessed as having probable or possible relationship to the trial product. Of 5 adverse events experienced following Actrapid t = 0, 1 was assessed as having probable or possible relation to the trial product. Two of the 7 events experienced following Actrapid t = -30 were assessed as having probable or possible relation to the trial product. The most common adverse events were: rhinitis and hypoglycaemia.
- symptoms of hypoglycaemia on study days were generally infrequent
- there were no clinically significant changes in any of the safety parameters

CONCLUSIONS

- analysis of the glucose endpoints demonstrated an improved postprandial glucose control when comparing IAsp with Act $_0$, based on a statistically significantly smaller $EXC_{(glu)}$ and supported by a statistically significantly lower $C_{max(glu)}$
- no difference in glucose endpoints between IAsp and Act $_{30}$ could be demonstrated
- no safety concerns about IAsp were raised by this trial

Ethics Committee approval and written informed consent were obtained prior to starting the trial. The trial was conducted in accordance with the Helsinki Declaration and with Good Clinical Practice.

TITLE OF TRIAL
A single centre randomised, open labelled, two-period cross-over trial in healthy subjects investigating the effect of NPH insulin on the pharmacokinetics of insulin aspart when administered as two injections or mixed prior to injection.

INVESTIGATOR

TRIAL CENTRE
[ ]

PUBLICATIONS
None

TRIAL PERIOD	DEVELOPMENT PHASE
27 February 1998 to 30 April 1998	I

OBJECTIVES

Primary

- to test the equivalence between two injection regimens by comparing the pharmacokinetics of insulin aspart when administered subcutaneously with NPH insulin as two separate injections or when mixed with NPH insulin prior to injection in healthy subjects.

Secondary

- to explore the equivalence between two injection regimens by comparing the pharmacokinetics of NPH insulin when administered with insulin aspart as two separate injections or when mixed prior to injection in healthy subjects.
- to evaluate the serum glucose profile following subcutaneous administration of insulin aspart and NPH insulin in healthy subjects, when injected separately or when mixed prior to injection.

METHODOLOGY
A randomised, open labelled, two-period cross-over design in which healthy male and female subjects received a single dose of insulin aspart and NPH insulin, as two separate injections and as a mixed injection, separated by a washout period of at least 7 days.

NUMBER OF SUBJECTS
- Twenty eight (28) subjects (14 males and 14 females) were randomised and enrolled in the trial.
- Twenty four (24) subjects (12 males and 12 females) completed the trial and four were withdrawn due to adverse events.
- Twenty eight (28) subjects were included in the safety analysis and twenty one (21) subjects (10 males and 11 females) were included in the efficacy analysis as three subjects were excluded due to a dosing failure.

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION
Healthy, non-smoking, male (aged 18 to 50 years) and female (aged 18 to 40 years) subjects with a body mass index of <29 kg/m^2. Written informed consent was obtained before inclusion of a subject in the study.

TEST REGIMEN, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER
A mixture of insulin aspart (0.06 U/kg BW) and NPH human isophane insulin (0.14 IU/kg BW), mixed approximately 3 minutes prior to dosing, was administered by a single subcutaneous injection into a skinfold perpendicular to the anterior abdominal wall by use of a NovoPen®; batch numbers C97004 (insulin aspart) and GQ50126 (NPH insulin).

REFERENCE REGIMEN, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

Two separate injections of insulin aspart (0.06 U/kg BW) and NPH human isophane insulin (0.14 IU/kg BW) were administered by subcutaneous injection into a skinfold perpendicular to the anterior abdominal wall by use of a NovoPen®; batch numbers C97004 (insulin aspart) and GQ50126 (NPH insulin).

DURATION OF TREATMENT

Single doses of insulin aspart and NPH insulin on two study days, separated by a washout period of at least 7 days between the two study days.

CRITERIA FOR EVALUATION – EFFICACY

The primary efficacy parameters were:

MRT_{IAsp}, the mean residence time of serum insulin aspart in the time interval 0 to 24 hours after injection.

$AUC_{IAsp,\ 0-8h}$, the area under the serum insulin aspart concentration curve in the time interval 0 to 8 hours after injection.

The secondary efficacy parameters were:

$AUC_{IAsp,\ 0-24h}$, the area under the serum insulin aspart concentration curve in the time interval 0 to 24 hours.

$C_{max\,IAsp}$, the maximal serum insulin aspart concentration in the time interval 0 to 24 hours.

$t_{max\,IAsp}$, time to maximal serum insulin aspart concentration in the interval 0 to 24 hours.

$AUC_{hum,\ 0-24h}$, the area under the serum human insulin concentration curve in the time interval 0 to 24 hours after injection.

$C_{max\,hum}$, the maximal serum human insulin concentration in the time interval 0 to 24 hours.

$t_{max\,hum}$, time to maximal serum human insulin concentration in the interval 0 to 24 hours.

$AOC_{Gluc,\ 0-24h}$, the area above the serum glucose concentration curve and below baseline value in the time interval 0 to 24 hours.

$C_{min\,Gluc}$, the minimal serum glucose concentration in the time interval 0 to 8 hours.

$t_{min\,Gluc}$, time to minimal serum glucose concentration in the time interval 0 to 8 hours.

The exploratory efficacy parameters for insulin aspart were:

$AUC_{IAsp,\ 0-\infty}$, the area under the serum insulin aspart concentration curve in the time interval 0 to infinity.

$\lambda_{z\,IAsp}$, elimination rate constant of serum insulin aspart.

$t_{½\,IAsp}$, elimination half life of serum insulin aspart.

CRITERIA FOR EVALUATION – SAFETY

Physical examination, vital signs, haematology, biochemistry and urinalysis laboratory analyses, 12-lead ECG, adverse events and blood glucose measurements.

STATISTICAL METHODS

All endpoints, except $t_{max\,IAsp}$, $t_{max\,hum}$ and $t_{min\,Gluc}$, were logarithmically transformed (using the natural logarithm) before they were subject to analysis of variance.

The statistical model included the fixed treatment (injection regimen) effect, fixed sequence effect, fixed visit effect, fixed gender effect, random subject (within sequence) effect and error term. This model is standard for equivalence studies.

For the logarithmically transformed endpoints, the mean difference on the log scale between the two injection regimens was estimated and the 90% confidence interval for the mean difference was calculated. The confidence intervals were then detransformed to give a 90% confidence interval for the ratio. The mean difference was also detransformed to give the geometric mean ratio.

The endpoints $t_{max\,IAsp}$, $t_{max\,hum}$ and $t_{min\,Gluc}$ were analysed nonparametrically using the Wilcoxon Signed Rank test on the paired differences. A 90% confidence interval of the median difference was constructed, where the median was the Hodges-Lehman estimator.

In order to declare equivalence, the confidence interval for the primary endpoints had to be completely contained in the ——— , interval.

EFFICACY RESULTS

The results of this trial confirmed that insulin aspart was rapidly absorbed when administered as a mixed or separate injection, with $C_{max(IAsp)}$ occurring at approximately 1 hour post-dose, and that its duration of action was short, with serum concentrations almost returning to baseline by approximately 6 hours post-dose.

Based on the analysis of the primary pharmacokinetic endpoints for insulin aspart, $MRT_{(IAsp)}$ and $AUC_{(IAsp,\ 0\text{-}8\ h)}$, equivalence was established between the separate injection compared to the mixed injection regimens (90% CI within the ——% regulatory limits), when insulin aspart was mixed with NPH insulin approximately 3 minutes prior to subcutaneous injection in healthy subjects.

The analysis results of the primary insulin aspart endpoints are shown in the table below:

Analysis Results of Primary Insulin Aspart Endpoints (n=21)

Mixed injection versus separate injections	Estimated Geometric Mean Ratio	Lower 90% C.I.	Upper 90% C.I.
Insulin aspart AUC(0-8 h)	0.96	0.85	1.09
Insulin aspart MRT	1.11	1.03	1.20

The results from the secondary insulin aspart endpoint $AUC_{(IAsp,\ 0\text{-}24\ h)}$ demonstrated equivalence between the two injection regimens, whereas the secondary insulin aspart endpoint $C_{max(IAsp)}$ did not show equivalence. Statistically significant differences were shown between the two injection regimens for $C_{max(IAsp)}$ and $t_{max(IAsp)}$, with the estimate of $C_{max(IAsp)}$ being approximately 17% lower and the estimate of $t_{max(IAsp)}$ being approximately 10 minutes earlier for the mixed injection compared to the separate injection regimen.

Analysis of the secondary NPH insulin endpoints, $AUC_{(NPH,\ 0\text{-}24\ h)}$ and $C_{max(NPH)}$, did not show equivalence between the two injection regimens. There were no statistically significant differences between the injection regimens for $AUC_{(NPH,\ 0\text{-}24\ h)}$ and $t_{max(NPH)}$, whereas there was a statistically significant difference between the injection regimens for $C_{max(NPH)}$, with the estimate of $C_{max(NPH)}$ being approximately 29% higher following the mixed injection compared to the separate injection regimens.

The serum glucose response to the two injection regimens, as assessed by the secondary glucose endpoints, $AOC_{(gluc,\ 0\text{-}24\ h)}$ and $C_{min(gluc)}$, supported the primary insulin aspart endpoints results in that the mixed injection and the separate injection regimens are equivalent and, furthermore, no significant difference between the separate and mixed injection regimens was revealed for the secondary glucose endpoint $t_{max(gluc)}$.

SAFETY RESULTS

There were no serious adverse events reported during the trial. The majority of adverse events were mild in severity. Three treatment-emergent adverse events (dizziness, palpitations and syncope) were considered to be severe. Three subjects were withdrawn from the trial due to adverse events considered to be unrelated to the study drug and one subject was withdrawn from the trial due to symptoms of hypoglycaemia (increased sweating, dizziness and palpitations) which were considered to be related to the study drug. The frequency and type of adverse events were similar for the two injection regimens with the majority of reported adverse events being related to hypoglycaemia.

There were no clinically significant findings for clinical laboratory tests, vital signs and ECGs during the trial. There was one clinically significant finding for the physical examinations experienced by one subject prior to dosing which resulted in the withdrawal of this subject from the trial.

CONCLUSIONS

- Based on the primary pharmacokinetic endpoints for insulin aspart, $MRT_{[IAsp]}$ and $AUC_{[IAsp,\ 0\text{-}4h]}$, equivalence was established between the separate injection and the mixed injection regimens, when insulin aspart was mixed with NPH insulin approximately 3 minutes prior to subcutaneous injection in healthy subjects.
- The secondary insulin aspart and NPH insulin endpoints, $C_{max[IAsp]}$, $t_{max[IAsp]}$, $C_{max[NPH]}$ and $AUC_{[NPH,\ 0\text{-}24\ h]}$, indicated that a small fraction of insulin aspart was exchanged with human insulin from NPH insulin, when the two types of insulin were mixed prior to injection.
- The serum glucose response to the two injection regimens as assessed by the secondary glucose endpoints, $AOC_{[glu,\ 0\text{-}24\ h]}$ and $C_{max[glu]}$, supported the primary insulin aspart endpoints results in that the two injection regimens were equivalent and, furthermore, no statistically significant difference was revealed for the secondary glucose endpoint $t_{max[glu]}$. Thus, the results demonstrated that the exchange was not clinically relevant at the mixing-to-injection-time studied.
- The frequency and type of adverse events were similar for the two injection regimens with the majority of reported adverse events being related to hypoglycaemia. There were no safety concerns following the mixed or separate injections.
- Overall, the results of the present trial support the possibility of self-mixing insulin aspart with NPH insulin in the syringe, if injection is performed immediately after mixing.

The trial was conducted in accordance with the Declaration of Helsinki and with Good Clinical Practice.

APPEARS THIS WAY
ON ORIGINAL